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Notes:

1) Segmented by Journals which less than 50 references
2) Journals are listed alphabetically from A to Z
3) Journal names are abbreviated (Please see journal list for the abbreviation)

Acta Cytol (1)


OBJECTIVE: To compare the performance of human papillomavirus (HPV) DNA detection by polymerase chain reaction (PCR) and Hybrid Capture II (HCII) test (Digene, Gaithersburg, Maryland, U.S.A.) in residual cells left in the collection vials of the DNACitoliq system (Digene Brasil, São Paulo, Brazil). STUDY DESIGN: A series of 263 cervical samples collected for liquid-based cytology with the DNACitoliq system was tested for oncogenic HPV types first with HCII and subsequently with PCR. After DNA purification with GFX Genomic Blood DNA Purification Kit (Amersham, Piscataway, New Jersey, U.S.A.), PCR was performed using AmpliTaq Gold DNA polymerase (Applied Biosystems). PGMY09/11 L1 consensus primers and GH20/PCO4 primers for human beta-globin target were coamplified. RESULTS: Altogether, 260 samples were positive for beta-globin, and 3 negative ones were excluded from the analysis. PCR and HCII yielded concordant results in 199 cases (76.5%) (102 positive and 97 negative), with Cohen's kappa of 577 (95% CI.477-.677) and weighted kappa of 733 (95% CI.659-.791). HPV prevalence in different categories of cytologic abnormalities was practically identical with HCII and PCR assays (P=.989). Among the 61 (23.5%) discrepant cases, 28 samples were HCII+/PCR- cases. Of these, 27 of 28 samples showed a low viral load, and 1 had an intermediate viral load.

CONCLUSION: The data suggest that residual material from the DNACitoliq system adequately preserves HPV DNA for detection by HCII and PCR, with performance similar to that of specimen transport medium.

Acta Tropica (6)

http://www.sciencedirect.com/science/article/B6T1R-476YG0D-2M/2/4f22d3380220b2c2ddbf121a6580ad6

We have developed a sensitive and specific method to identify Trypanosoma brucei gambiense using the polymerase chain reaction (PCR) to amplify the gene encoding variant surface glycoprotein (VSG) Antat 11.17. The test was capable of distinguishing T. b. gambiense from T. b. brucei in most foci of gambian sleeping sickness and gave positive results with previously well-characterised Type I T. b. gambiense stocks from Ivory Coast, Nigeria, Cameroon, Congo, Zaire and Sudan. The test gave negative results with T. b. rhodesiense from Zambia, Kenya and Uganda, virulent or Type II T. b. gambiense from Ivory Coast and T. b. brucei stocks from East and West Africa. The test was modified for colorimetric detection in dot blot format by using nested biotinylated primers in a two-step reaction. Comparison of DNA sequences of VSG genes from T. b. gambiense and other T. brucei ssp. stocks showed a high level of homology, suggesting recent gene flow.


http://www.sciencedirect.com/science/article/B6T1R-4384P6V-2/2/dc4720074b590ef3c92972f1135a16bb

Toxocara canis is an ascarid nematode parasite of canids. Larvae infect a wide range of accidental hosts including humans, in whom they are the aetiologic agent of visceral and ocular Larva migrans. The labile surface coat of T. canis larvae consists of a family of mucin glycoproteins termed TES-120, for which the cDNAs have recently been cloned. In this paper, we describe the identification of a novel cDNA (Tc-muc-5) encoding an apomucin by expression screening of a cDNA library with antiserum raised to T. canis excretory/secretory products, and compare the predicted Tc-MUC-5 protein with those of other T. canis mucins (Tc-MUC-1-Tc-MUC-4) that include the TES-120 surface coat glycoproteins. Tc-MUC-5 has both a larger open reading frame and a more divergent sequence than the other T. canis mucins. It contains a putative signal peptide followed by two six-cysteine (SXC) domains, an extended threonine-rich central mucin core domain and two C-terminal SXC domains. Amino acid composition analysis of secreted TES-120 glycoproteins revealed a distinct lack of lysine residues; while this finding is in agreement with the primary sequences of Tc-MUC-1-Tc-MUC-4, Tc-MUC-5 is conspicuous by its relative abundance of lysines (6.7%), suggesting that this protein is not part of the TES-120 family of surface coat proteins.


http://www.sciencedirect.com/science/article/B6T1R-46WWBK3-1/2/779256dfc9f8a53fd5a1b34b9f9e45f4

The diversity of Plasmodium falciparum clones and their role in progression from asymptomatic to symptomatic condition in children have been investigated. Attempts to identify whether particular parasite genotypes were associated with the development of clinical symptoms have been made. A cohort of 34 initially asymptomatic parasitaemic children aged 1-5 years were followed daily for 31 days. Clinical examinations were made each day for signs and symptoms of clinical malaria,
followed by parasitological investigation. Nineteen children developed symptoms suggestive of clinical malaria during this period. Daily blood parasite samples from 13 children who developed clinical malaria symptoms and 7 who remained asymptomatic were genotyped by PCR-amplification of the polymorphic regions of the merozoite surface proteins 1 and 2 (MSP1 and MSP2) and the glutamate rich protein (GLURP) genes. Infections were found to be highly complex in both groups of children. Every isolate examined from both groups had a mixture of parasite clones. Daily changes were observed in both parasite density and genotypic pattern. The mean number of genotypes per individual was estimated at 4.9 and 2.7 for asymptomatic and symptomatic groups of children, respectively. Analysis of allele frequency distributions showed that these differed significantly for the MSP1 locus only.


http://www.sciencedirect.com/science/article/B6T1R-3SY8DCX-B/2/a963542d2f190bf961794fcede0b27dd13

We compared the palpal ratio method with the polymerase chain reaction (PCR) to distinguish between Anopheles gambiae s.s. and A. melas. At the end of the rainy season of 1995, female mosquitoes were collected indoors in the Antula area of Bissau, Guinea Bissau. A subsample of 354 mosquitoes were identified first with PCR and then with the palpal ratio method (study A). Subsequently, another 195 mosquitoes were identified first with the palpal ratio method and then with PCR (study B). The highest percentage (100%, n=16) of correctly identified A. melas was obtained at the palpal ratio cut-off point of 0.83. However, at this point 4.0% (14/347) and 11.3% (21/186) of the A. gambiae were erroneously identified as A. melas in study A and B, respectively. This suggests that the palpal ratio method is not sufficiently reliable to distinguish between A. gambiae and A. melas from the Bissau area.


http://www.sciencedirect.com/science/article/B6T1R-44BFJ67-F/2/b66c307751560a0d99ef800224e40243

Species within the genus Bulinus are responsible for transmission of schistosomes within the Schistosoma haematobium group. In order to provide a molecular insight into the species relationships within the genus, genetic variation between species representing the four species groups was assayed by Polymerase Chain Reaction (PCR) amplification of the ribosomal Internal Transcribed Spacer (ITS) region followed by Restriction Fragment Length Polymorphism (RFLP) analysis of this product with six restriction enzymes. This PCR-RFLP methodology detected considerable variation within the ITS region indicating that restriction profiles will be useful as genetic markers for identification purposes. The complete ITS1 spacer was sequenced for B. globosus, B. cernicus and B. truncatus. There were numerous nucleotide differences between taxa mainly insertions and deletions. Nucleotide divergence was calculated between species from the restriction profiles: the B. truncatus/tropicus complex and B. reticulatus group were most similar which were in turn more closely related to the B. africanaus group than to the B. forskalii group. The nucleotide divergence between the species groups is substantial and questions the placement of these groups within the same genus.
Diagnostic differentiation of pathogenic Entamoeba histolytica from non-pathogenic Entamoeba dispar is of great clinical importance. We have developed and evaluated a new polymerase chain reaction (PCR) assay (haemo-PCR) based on the novel E. histolytica hemolysin gene HLY6. The specificity of this assay was confirmed by analyzing different Entamoeba species, faeces samples, human and bacterial DNA, and digestion of amplification products with appropriate restriction enzymes. The sensitivity was confirmed by serial dilutions of E. histolytica HM-1:IMSS DNA in the excess of human DNA. Totally, 45 clinical samples were analyzed by the haemo-PCR assay including amoebic liver abscess (ALA) fluids from 23 patients suspected for amoebiasis, four faeces samples containing E. histolytica and E. dispar, and positive and negative controls. The results were compared with those obtained with PCRs for cystein-rich surface protein (P30) and small subunit ribosomal RNA (ssu rRNA) genes. The haemo-PCR gave a positive result in 18 (89%) ALA fluids compared with 14 (77%) and five (28%) by PCR for p30, and ssu rRNA, respectively. PCR products were obtained only from specimens containing E. histolytica DNA. The haemo-PCR assay was therefore found to be a valuable diagnostic tool for identification of E. histolytica infections both in faeces and ALA samples.

Addictive Behaviors (1)


AimsA linkage of certain alleles of the tryptophan hydroxylase (TPH) intron 7 A218C polymorphism to suicidality and antisocial behaviour has been described. The aim of our study was to find any association between dimensions of the Temperament and Character Inventory (TCI) indicating impulsivity and the TPH polymorphism alleles in unselected alcohol-dependent patients and age-matched controls.MethodsWe examined 159 alcohol-dependent patients and 161 controls with the TCI and genotyped them for the TPH intron 7 A218C polymorphism alleles.ResultsAlthough homozygous TPH genotypes were found more often in alcohol-dependent patients than in controls, an association between TCI dimensions and TPH alleles was not observed in the complete sample. Alcohol-dependent patients, however, scored significantly higher for harm avoidance (HA) and lower for self-directedness (SD) than controls regardless of TPH genotype. Among controls, for those with the A/A genotype, harm avoidance was as high as in the group of alcohol-dependent patients, persistence (P) in that genotype was significantly lower than for all other genotypes in the patient and control group.ConclusionEven if there is no association between TCI dimensions and TPH genotype in our sample, hints to nonspecific psychopathology in connection with the A/A genotype are found.

The biological effects of high LET charged particles are a subject of great concern with regard to the prediction of radiation risk in space. In this report, mutagenic effects of high LET charged particles are quantitatively measured using primary cultures of human skin fibroblasts, and the spectrum of induced mutations are analyzed. The LET of the charged particles ranged from 25 KeV/[mu]m to 975 KeV/gmm with particle energy (on the cells) between 94 - 603 MeV/u. The X-chromosome linked hypoxanthine guanine phosphoribosyl transferase (hprt) locus was used as the target gene. Exposure to these high LET charged particles resulted in exponential survival curves; whereas, mutation induction was fitted by a linear model. The Relative Biological Effect (RBE) for cell-killing ranged from 3.73 to 1.25, while that for mutant induction ranged from 5.74 to 0.48. Maximum RBE values were obtained at the LET of 150 keV/[mu]m. The inactivation cross-section ([alpha]i) and the action-section for mutant induction ([alpha]m) ranged from 2.2 to 92.0 [mu]m2 and 0.09 to 5.56 x 10-3 [mu]m2, respectively. The maximum values were obtained by 56Fe with an LET of 200 keV/[mu]m. The mutagenicity ([alpha]m/[alpha]i) ranged from 2.05 to 7.99 x 10-5 with the maximum value at 150 keV/[mu]m. Furthermore, molecular analysis of mutants induced by charged particles indicates that higher LET beams are more likely to cause larger deletions in the hprt locus.


Dormant spores of the fern Ceratopteris richardii were flown on Shuttle mission STS-93 to evaluate the effects of micro-g on their development and on their pattern of gene expression. Prior to flight the spores were sterilized and sown into one of two environments: (1) Microscope slides in a video-microscopy module; and (2) Petri dishes. All spores were then stored in darkness until use. Spore germination was initiated on orbit after exposure to light. For the spores on microscope slides, cell level changes were recorded through the clear spore coat of the spores by video microscopy. After their exposure to light, spores in petri dishes were frozen in orbit at four different time points during which on earth gravity fixes the polarity of their development. Spores were then stored frozen in Biological Research in Canister units until recovery on earth. The RNAs from these cells and from 1-g control cells were extracted and analyzed on earth after flight to assay changes in gene expression. Video microscopy results revealed that the germinated spores developed normally in microgravity, although the polarity of their development, which is guided by gravity on earth, was random in space. Differential Display-PCR analyses of RNA extracted from space-flown cells showed that there was about a 5% change in the pattern of gene expression between cells developing in micro-g compared to those developing on earth.

http://www.sciencedirect.com/science/article/B6T40-3XR8CHH/2/de85fc7dd6b426debe9e6138cf6533a3ad

Deficiency of mitochondrial aldehyde dehydrogenase (ALDH2) has been previously reported in South American Indians. We therefore assayed five individuals from each of five South American populations (Quechua, Karitiana, Ticuna, Surui, Guahiba), and two North American populations (Maya and Moskoke) for the presence of the Oriental ALDH22 variant. These samples were also surveyed for other alleles altering ALDH2 function. Allele-specific amplification assay (ASA) did not detect the ALDH22 allele in any of the New World populations studied. The entire coding sequence of the ALDH2 cDNA was enzymatically amplified in partially overlapping fragments. Each fragment was digested using restriction endonucleases and subfragments 148-285 b.p. in length were analyzed by the single-stranded conformation polymorphism (SSCP) technique. No variants were detected within the coding region of the ALDH2 gene in any of the seven American Indian populations. Three potentially correct explanations for these results are suggested. First, an ALDH2 polymorphism is present but undetectable by SSCP; second, none of the studied individuals were ALDH2 negative; third, the polymorphism occurs beyond the coding region of ALDH2 gene.


http://www.sciencedirect.com/science/article/B6T40-485N5G85/2/97ef7b70fe9add3d6f38d2c89ba0f1e

Results of recent studies have indicated an association between voluntary alcohol intake and activities of [kappa]-opioid receptor systems in animal models. We assessed the possibility that genetic differences observed in alcohol preference among mouse strains are related to possible polymorphisms of the [kappa]-opioid receptor gene (Oprk1). We compared DNA sequences of the coding region and the promoter/regulatory region of Oprk1 among C57BL/6ByJ (B6, alcohol-preferring), BALB/cJ (alcohol-avoiding), CXB1 (alcohol-avoiding), and six B6.C and B6.I Recombinant QTL Introgression (RQI) strains, which carry ~3% of the donor BALB/cJ genome in the background B6 genome and showed various alcohol preferences. Although there were no sequence differences in the coding region, BALB/cJ had a single nucleotide polymorphism (SNP) in the promoter region, which was not detected in other strains. The results indicate that the difference in alcohol preference between B6 and BALB/cJ is not correlated with polymorphisms of Oprk1. However, results of further studies comparing Oprk1 mRNA expression between B6 and BALB/cJ showed that Oprk1 expression is regulated differently in these strains. Also, DBA/2J mice (alcohol-avoiding) showed expression of Oprk1 mRNA subtypes (alternatively spliced) different from B6 and BALB/cJ mice. Search of the Celera Genomics database indicated that DBA/2J had several SNP sites in the promoter/regulatory regions, which might explain the different expression of Oprk1 mRNA subtypes in this strain. The strain-dependent variation in the expression of alternatively spliced genes can be a significant source of phenotypic variation of complex traits such as alcohol preference.
Ethanol preference, a component of alcoholism, has been known for four decades to differ greatly between C57BL/6 and BALB/c inbred mouse strains. For mapping quantitative trait loci (QTLs) that affect ethanol preference, we used a set of B6.C Recombinant QTL Introgression (RQI) strains, which carry about 5% of the donor BALB/cJ (C) genome on a C57BL/6ByJ (B6) background. After characterizing males of the progenitor and RQI strains for variations in ethanol preference, we scanned their genome for polymorphisms at 244 dinucleotide-repeat marker loci known to differ between B6 and C. Because of the introgression of BALB/c-type QTLs onto the B6 background, some strains showed ethanol preference significantly lower or higher than that of the background strain, suggesting that genetic interaction between ethanol preference QTLs and the background can be operative. The genomic region showing the strongest influence on ethanol preference was on mouse chromosome 15, and corresponds to human chr.12 q11-q13.

In our present genetic study to map Quantitative Trait Loci (QTLs) for alcohol-related behaviors, we used 44 B6.C and 36 B6.I inbred congenic Recombinant QTL Introgression (RQI) mouse strains of the b5i7 series carrying genes of BALB/cJ (C) or CXBI (I) origin on C57BL/6ByJ (B6) genetic background. Ethyl alcohol consumption (EAC) was measured in adult males, and chromosomes 1, 2, 3, 9, and 15 were scanned with polymorphic microsatellite markers. In the B6.C set of strains, multiple regression analysis yielded a model with three microsatellite markers, which explained 32% of the genetic variance (p=0.0006). The two markers with the highest significance levels in the model, D1Mit167 and D2Mit74, have been mapped to chromosome regions close to the gene opioid receptor kappa 1 (chr. 1) and opioid receptor kappa 3 (chr. 2), respectively. The results of this gene-mapping study suggest that genetic polymorphisms in kappa opioid receptors may contribute to genetic predisposition to voluntary alcohol-drinking behavior.
(ALDH2) Glu487Lys were genotyped by a duplex polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP), which allows DNA amplification with one-tube PCR including eight primers, and subsequent electrophoresis. Methods: Several PCR conditions were tested to establish the optimal conditions for distinguishing the allele-specific bands for the two polymorphisms. Under the optimal PCR conditions, 454 Japanese health check-up examinees were genotyped. Results: The allele-specific bands were successfully amplified under the optimal conditions of the duplex PCR-CTPP. The genotype distributions were within the Hardy-Weinberg equilibrium. The bands produced by the duplex PCR-CTPP genotyping were clearer than those produced by PCR-CTPP, conducted solely for ADH2. Conclusions: ADH2 Arg47His and ALDH2 Glu487Lys were successfully genotyped by this newly developed duplex PCR-CTPP, an inexpensive and time-saving genotyping tool, which will be useful in epidemiological studies on alcoholism, as well as risk estimation of alcohol-related diseases.


http://ajpcell.physiology.org/cgi/content/abstract/282/4/C768

The cardiac L-type calcium current (ICa) can be modified by activation of protein kinase C (PKC). However, the effect of PKC activation on ICa is still controversial. Some studies have shown a decrease in current, whereas other studies have reported a biphasic effect (an increase followed by a decrease in current or vice versa). A possible explanation for the conflicting results is that several isoforms of PKC with opposing effects on ICa were activated simultaneously. Here, we examined the influence of a single PKC isoform (PKC-[beta]II) on L-type calcium channels in isolation from other cardiac isoforms, using a transgenic mouse that conditionally expresses PKC-[beta]II. Ventricular cardiac myocytes were isolated from newborn mice and examined for expression of the transgene using single cell RT-PCR after ICa recording. Cells expressing PKC-[beta]II showed a twofold increase in nifedipine-sensitive ICa. The PKC-[beta]II antagonist LY-379196 returned ICa amplitude to levels found in non-PKC-[beta]II-expressing myocytes. The increase in ICa was independent of Cav1.2-subunit mRNA levels as determined by quantitative RT-PCR. Thus these data demonstrate that PKC-[beta] is a potent modulator of cardiac L-type calcium channels and that this specific isoform increases ICa in neonatal ventricular myocytes.


http://ajpcell.physiology.org/cgi/content/abstract/286/4/C779

Angiotensin II (ANG II) has been etiologically linked to vascular disease; however, its role in the alterations of endothelial function that occur in vascular disorders is not completely understood. Matrix metalloproteinases (MMPs) and proinflammatory cytokines are involved in the pathological remodeling of blood vessels that occurs in vascular disease. In this study we evaluated the effects of ANG II on tumor necrosis factor (TNF)-{alpha} and MMP-2 production in endothelial cells. Human umbilical vein endothelial cells (HUVECs) were stimulated with ANG II (0.1-10
{micro}M) for 24 h, in the presence or absence of antagonists of ANG II type 1 (AT1R) and type 2 (AT2R) receptors, and the production and release of TNF-(alpha) and MMP-2 were assessed. ANG II increased TNF-(alpha) mRNA and protein expression and the release of bioactive TNF-(alpha). Moreover, ANG II induced MMP-2 release and reduced the secretion of tissue inhibitor of MMP (TIMP)-2 from endothelial cells. To elucidate whether endogenous TNF-(alpha) could mediate the effects of ANG II on MMP-2 release, cells were pretreated with anti-TNF-(alpha) neutralizing antibodies or pentoxifylline (an inhibitor of TNF-(alpha) synthesis). TNF-(alpha) inhibition prevented the secretion of MMP-2 induced by ANG II. Furthermore, AT1R antagonism with candesartan prevented the formation of MMP-2 and TNF-(alpha) and the reduction of TIMP-2 induced by ANG II. These results indicate that ANG II, via AT1R, modulates the secretion of TNF-(alpha) and MMP-2 from endothelial cells and that TNF-(alpha) mediates the effects of ANG II on MMP-2 release.


http://ajpcell.physiology.org/cgi/content/abstract/285/6/C1445

Purinergic inhibition of Na-K-Cl cotransport has been noted in various renal epithelial cells derived from the collecting tubule, including Madin-Darby canine kidney (MDCK) cells. In recent studies, we have observed purinergic inhibition of Na-K-Cl cotransport in C11-MDCK subclones ((alpha)-intercalated-like cells). Interestingly, Na-K-Cl cotransport activity was also detected in C7-MDCK subclones (principal-like cells) but was not affected by ATP. In this investigation, we have transfected the human Na-K-Cl cotransporter (huNKCC1) in both C11 and C7 cells to determine whether these differences in NKCC regulation by ATP were due to cell-specific purinoceptor signaling pathways or to cell-specific isoforms/splice variants of the transporter. In both cell lines, we found that endogenous as well as huNKCC1-derived cotransport activity was restricted to the basolateral side. In addition, we were able to show that extracellular application of 100 {micro}M ATP or 100 {micro}M UTP abolished NKCC activity in both mock- and huNKCC1-transfected C11 cells but not in mock- and huNKCC1-transfected C7 cells; in C11 cells, intriguingly, this inhibition was not affected by inhibitors of RNA and protein synthesis and occurred even though expression levels of UTP-sensitive P2Y2-, P2Y4-, and P2Y6-purinoceptors were not different from those observed in C7 cells. These results suggest that C11 cells express an undetermined type of UTP-sensitive P2-purinoceptors or a unique P2Y-purinoceptor-triggered signaling cascade that leads to inhibition of NKCC1.


http://ajpcell.physiology.org/cgi/content/abstract/288/2/C416

The aim of the present study was to determine the distribution of monocarboxylate transporter (MCT) subtypes 1-4 in the various structures of the rat eye by using a combination of conventional and real-time RT-PCR, immunoblotting, and immunohistochemistry. Retinal samples expressed mRNAs encoding all four MCTs. MCT1 immunoreactivity was observed in photoreceptor inner segments, Muller cells, retinal capillaries, and the two plexiform layers. MCT2 labeling was concentrated in the inner and outer plexiform layers. MCT4 immunolabeling was present only in the inner retina, particularly in putative Muller cells, and the plexiform layers. No MCT3 labeling could be observed. The retinal pigment epithelium (RPE)/choroid expressed high levels of MCT1 and MCT3 mRNAs but lower levels of MCT2 and MCT4 mRNAs. MCT1 was localized to the apical and MCT3 to the basal membrane of the RPE, whereas MCT2 staining was faint. Although MCT1-MCT4 mRNAs were all detectable in iris and ciliary body samples, only
MCT1 and MCT2 proteins were expressed. These were present in the iris epithelium and the nonpigmented epithelium of the ciliary processes. MCT4 was localized to the smooth muscle lining of large vessels in the iris-ciliary body and choroid. In the cornea, MCT1 and MCT2 mRNAs and proteins were detectable in the epithelium and endothelium, whereas evidence was found for the presence of MCT4 and, to a lesser extent, MCT1 in the lens epithelium. The unique distribution of MCT subtypes in the eye is indicative of the pivotal role that these transporters play in the maintenance of ocular function.


http://ajpcell.physiology.org/cgi/content/abstract/287/4/C971

Primary cultures of granule cells (GC) from rat cerebellar cortex were used to determine whether bioelectric activity, via a Ca2+/calmodulin-dependent kinase (CaMK) signaling cascade, modulates expression and exon selection in the inositol trisphosphate receptor type 1 (IP3R1). IP3R1 contains or lacks three exons (S1, S2, and S3) that are regulated in a regionally and temporally specific manner. The neuronal, or long, form of IP3R1 is distinguished from peripheral tissues by inclusion of the S2 exon. Although previous studies indicated that IP3R1 are undetectable in the cerebellar granular layer in vivo, receptor protein and mRNA are induced in cultured GC grown in medium supplemented with 25 mM KCl or NMDA, two trophic agents that promote long-term survival, compared with GC grown in 5 mM KCl. IP3R1 induction in response to 25 mM KCl or NMDA is attenuated by coaddition of voltage-sensitive calcium channel or NMDA receptor antagonists, respectively. Actinomycin D, CaMK, and calcineurin antagonists likewise suppress induction. Unlike the major variants of IP3R1 in Purkinje neurons, which lack S1 and S3, GC grown with trophic agents express mRNA containing these exons. Both neuronal types contain S2. Evidence obtained using mutant mice with Purkinje cell lesions, laser-microdissected GC neurons from slices, and explant cultures indicates that GC predominantly express the S1-containing variant of IP3R1 in vivo.


http://ajpcell.physiology.org/cgi/content/abstract/283/2/C587

ATP-sensitive K+ (KATP) channels are composed of pore-forming Kir6.x subunits and regulatory sulfonylurea receptor (SUR) subunits. SURs are ATP-binding cassette proteins with two nucleotide-binding folds (NBFs) and binding sites for sulfonylureas, like glibenclamide, and for channel openers. Here we report the identification and functional characterization of four novel splice forms of guinea pig SUR1. Three splice forms originate from alternative splicing of the region coding for NBF1 and lack exons 17 (SUR1[Delta]17), 19 (SUR1[Delta]19), or both (SUR1[Delta]17[Delta]19). The fourth (SUR1C) is a COOH-terminal SUR1-fragment formed by exons 31-39 containing the last two transmembrane segments and the COOH terminus of SUR1. RT-PCR analysis showed that these splice forms are expressed in several tissues with strong expression of SUR1C in cardiomyocytes. Confocal microscopy using enhanced green fluorescent protein-tagged SUR or Kir6.x did not provide any evidence for involvement of these splice forms in the mitochondrial KATP channel. Only SUR1 and SUR1[Delta]17 showed high-affinity binding of glibenclamide (Kd[approx] 2 nM in the presence of 1 mM ATP) and formed functional KATP channels upon coexpression with Kir6.2.
Epithelial cells of the epididymis and vas deferens establish an optimum luminal environment in which spermatozoa mature and are stored. This is achieved by active transepithelial transport of various ions including Cl[-] and H+. We investigated the localization of three closely related members of the ClC family, ClC-3, ClC-4, and ClC-5, in the epididymis and vas deferens. RT-PCR using mRNA isolated by laser capture microdissection (LCM)-detected ClC-3 and ClC-5 transcripts but did not detect any ClC-4-specific transcript. Western blot and immunofluorescence analysis demonstrated that ClC-3 and ClC-5 proteins are present in all regions of the epididymis and in the vas deferens. ClC-5 is expressed exclusively in H+-ATPase-rich cells (narrow and clear cells). Confocal microscopy showed that ClC-5 partially colocalizes with the H+-ATPase in the subapical pole of clear cells. ClC-3 is strongly expressed in the apical membrane of principal cells of the caput epididymidis and the vas deferens and is less abundant in principal cells of the body and cauda epididymidis. These findings are consistent with a potential role for ClC-3 in transepithelial chloride transport by principal cells and for ClC-5 in the acidification of H+-ATPase-containing vesicles in narrow and clear cells. ClC-5 might facilitate endosome trafficking in the epididymis, as has been proposed in the kidney.

Fish oils (FOs) have been noted to reduce growth and proliferation of certain tumor cells, effects usually attributed to the content of polyunsaturated fatty acids of the n-3 family, which are thought to modulate cellular signaling pathways. We investigated the influence of FO on cell cycle kinetics of cultured Chinese hamster ovary cells. Exponentially growing cells were labeled with 5-bromo-2'-deoxyuridine (BrdU) and analyzed by flow cytometry after 5-day treatment with exogenous fat. Bivariate BrdU-DNA analysis indicated slower progression through S phase and thus longer S phase duration time in FO- but not corn oil-treated or control cells. We hypothesize that FO treatment might interfere with spatial/temporal organization of replication origins. Therefore, we mapped the well-characterized replication origin ori-[beta] downstream of the dihydrofolate reductase gene with the nascent strand length assay. Three DNA marker segments with known positions relative to this origin were amplified by PCR. By quantitatively assessing DNA length of the fragments in all fractions containing these markers, the location of ori-[beta] was established. In control or corn oil-treated cells, the location of ori-[beta] was consistent with previous studies. However, in FO-treated cells, DNA replication appears to start from a new site located farther upstream from ori-[beta], suggesting a different replication initiation pattern. This study suggests novel mechanism(s) by which fats affect cell proliferation and DNA replication in mammalian cells.
Synaptotagmin I (Syt I), a low-affinity Ca\textsuperscript{2+}-binding protein, is thought to serve as the Ca\textsuperscript{2+} sensor in the release of neurotransmitter. However, functional studies on the calyx of Held synapse revealed that the rapid release of neurotransmitter requires only approximately micromolar [Ca\textsuperscript{2+}], suggesting that Syt I may play a more complex role in determining the high-affinity Ca\textsuperscript{2+} dependence of exocytosis. Here we tested this hypothesis by studying pituitary cells, which possess high- and low-affinity Ca\textsuperscript{2+}-dependent exocytic pathways and express Syt I. Using patch-clamp capacitance measurements to monitor secretion and the acute antisense deletion of Syt I from differentiated cells, we have shown that the rapid and the most Ca\textsuperscript{2+}-sensitive pathway of exocytosis in rat melanotrophs requires Syt I. Furthermore, stimulation of the Ca\textsuperscript{2+}-dependent exocytosis by cytosol dialysis with solutions containing 1 \(\text{micro}M\) [Ca\textsuperscript{2+}] was completely abolished in the absence of Syt I. Similar results were obtained by the preinjection of antibodies against the CAPS (Ca\textsuperscript{2+}-dependent activator protein for secretion) protein. These results indicate that synaptotagmin I and CAPS proteins increase the probability of vesicle fusion at low cytosolic [Ca\textsuperscript{2+}].


http://ajpcell.physiology.org/cgi/content/abstract/282/1/C172

First published September 5, 2001; 10.1152/ajpcell. 00048.2001.[---]Intestinal strictures are frequent in Crohn's disease but not ulcerative colitis. We investigated the expression of transforming growth factor (TGF)-[beta] isoforms by isolated and cultured primary human intestinal myofibroblasts and the responsiveness of these cells and intestinal epithelial cells to TGF-[beta] isoforms. Normal intestinal myofibroblasts released predominantly TGF-[beta]3 and ulcerative colitis myofibroblasts expressed both TGF-[beta]1 and TGF-[beta]3, whereas in myofibroblast cultures from fibrotic Crohn's disease tissue, there was significantly lower expression of TGF-[beta]3 but enhanced release of TGF-[beta]2. These distinctive patterns of TGF-[beta] isoform release were sustained through several myofibroblast passages. Proliferation of Crohn's disease myofibroblasts was significantly greater than that of myofibroblasts derived from normal and ulcerative colitis tissue. In contrast to cells from normal and ulcerative colitis tissue, neutralization of the three TGF-[beta] isoforms did not affect the proliferation of Crohn's disease intestinal myofibroblasts. Studies on the effect of recombinant TGF-[beta] isoforms on epithelial restitution and proliferation suggest that TGF-[beta]2 may be the least effective of the three isoforms in intestinal wound repair. In conclusion, the enhanced release of TGF-[beta]2 but reduced expression of TGF-[beta]3 by Crohn's disease intestinal myofibroblasts, together with their enhanced proliferative capacity, may lead to the development of intestinal strictures.


http://ajpcell.physiology.org/cgi/content/abstract/287/1/C22

Neonatal sciatic nerve injury is known to result in an extensive loss of lumbar motor neurons as well as the disappearance of their respective muscle fibers in the hindlimb musculature. The loss of motor neurons and muscle fibers can be prevented by immediate administration of target-derived neurotrophic factors to the site of injury. In the present study, we investigated the role of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) in the survival and maturation of a subset of motor neurons innervating the extensor digitorum longus (EDL) and tibialis anterior (TA) muscles. We have shown that combined administration of CNTF and BDNF prevented the loss of motor units after neonatal nerve injury and contributed to the maintenance
of muscle mass. Importantly, this combined neurotrophin regimen also prevented the disappearance of muscle fibers that express myosin heavy chain IIB (MyHC IIB) in both EDL and TA muscles 3 mo after neonatal sciatic nerve crush. In parallel studies, we observed a higher level of BDNF in EDL muscle during the critical period of development when motor neurons are highly susceptible to target removal. Given our previous findings that combined administration of CNTF with neurotrophin-3 (NT-3) or neurotrophin-4/5 (NT-4/5) did not result in the rescue of MyHC IIB fibers in EDL, the present results show the importance of muscle-derived BDNF in the survival and maturation of a subpopulation of motor neurons and of MyHC IIB muscle fibers during neonatal development of the neuromuscular system.


http://ajpcell.physiology.org/cgi/content/abstract/287/1/C125

We have previously reported that the hEAG K+ channels are responsible for the potential membrane hyperpolarization that induces human breast cancer cell progression into the G1 phase of the cell cycle. In the present study, we evaluate the role and functional expression of the intermediate-conductance Ca2+-activated K+ channel, hIK1-like, in controlling cell cycle progression. Our results demonstrate that hIK1 current density increased in cells synchronized at the end of the G1 or S phase compared with those in the early G1 phase. This increased current density paralleled the enhancement in hIK1 mRNA levels and the highly negative membrane potential. Furthermore, in cells synchronized at the end of G1 or S phases, basal cytosolic Ca2+ concentration ([Ca2+]i) was also higher than in cells arrested in early G1. Blocking hIK1 channels with a specific blocker, clotrimazole, induced both membrane potential depolarization and a decrease in the [Ca2+]i in cells arrested at the end of G1 and S phases but not in cells arrested early in the G1 phase. Blocking hIK1 with clotrimazole also induced cell proliferation inhibition but to a lesser degree than blocking hEAG with astemizole. The two drugs were essentially additive, inhibiting MCF-7 cell proliferation by 82% and arresting >90% of cells in the G1 phase. Thus, although the progression of MCF-7 cells through the early G1 phase is dependent on the activation of hEAG K+ channels, when it comes to G1 and checkpoint G1/S transition, the membrane potential appears to be primarily dependent on the hIK1-activity level.


http://ajpcell.physiology.org/cgi/content/abstract/284/4/C870

High mobility group box 1 (HMGB1) protein, a DNA binding protein that stabilizes nucleosomes and facilitates transcription, was recently identified as a late mediator of endotoxin lethality. High serum HMGB1 levels in patients with sepsis are associated with increased mortality, and administration of HMGB1 produces acute inflammation in animal models of lung injury and endotoxemia. Neutrophils occupy a critical role in mediating the development of endotoxemia-associated acute lung injury, but previously it was not known whether HMGB1 could influence neutrophil activation. In the present experiments, we demonstrate that HMGB1 increases the nuclear translocation of NF-[kappa]B and enhances the expression of proinflammatory cytokines in human neutrophils. These proinflammatory effects of HMGB1 in neutrophils appear to involve the p38 MAPK, phosphatidylinositol 3-kinase/Akt, and ERK1/2 pathways. The mechanisms of HMGB1-induced neutrophil activation are distinct from endotoxin-induced signals, because HMGB1 leads to a different profile of gene expression, pattern of cytokine expression, and kinetics of p38 activation compared with LPS. These findings indicate that HMGB1 is an effective
stimulus of neutrophil activation that can contribute to development of a proinflammatory phenotype in diseases characterized by excessively high levels of HMGB1.


http://ajpcell.physiology.org/cgi/content/abstract/00542.2004v1

Stimulation of ATP or adenosine receptors causes important physiologic changes in retinal pigment epithelial (RPE) cells that may influence their relationship to the adjacent photoreceptors. While RPE cells have been shown to release ATP, the regulation of extracellular ATP levels and the production of dephosphorylated purines is not clear. This study examined the degradation of ATP by RPE cells and the physiologic effects of the adenosine diphosphate (ADP) that results. ATP was readily broken down by both cultured human ARPE-19 cells and the apical membrane of fresh bovine RPE cells. The compounds ARL67156 and (beta)(gamma)mATP inhibited this degradation in both cell types. RT-PCR analysis of ARPE-19 cells found mRNA message for multiple extracellular degradative enzymes; ectonucleotide pyrophosphatase/ phosphodiesterase (eNPP)1, eNPP2 and eNPP3, the ectoATPase ecto-nucleoside triphosphate diphosphohydrolase (NTPDase)2, NTPDase3, and some message for NTPDase1. Considerable levels of ADP bathed RPE cells, consistent with a role for NTPDase2. ADP and ATP increased levels of intracellular Ca(2+). Both responses were inhibited by thapsigargin and P2Y1 receptor inhibitor MRS 2179. Message for both P2Y1 and P2Y12 receptors was detected in ARPE-19 cells. These results suggest that extracellular degradation of ATP in subretinal space can result in production of ADP. This ADP can stimulate P2Y receptors and augment Ca(2+) signaling in the RPE.


http://ajpcell.physiology.org/cgi/content/abstract/283/1/C347

In this study, we examined the role of the nuclear factor-(kappa)B (NF-(kappa)B)-inducing kinase (NIK) in distinct signaling pathways leading to NF-(kappa)B activation. We show that a dominant-negative form of NIK (dnNIK) delivered by adenoviral (Ad5dnNIK) vector inhibits Fas-induced (kappa)B[alpha] phosphorylation and NF-(kappa)B-dependent gene expression in HT-29 and HeLa cells. Interleukin (IL)-1[alpha]- and tumor necrosis factor-(alpha) (TNF-(alpha))-induced NF-(kappa)B activation and (kappa)B-dependent gene expression are inhibited in HeLa cells but not in Ad5dnNIK-infected HT-29 cells. Moreover, Ad5dnNIK failed to sensitize HT-29 cells to TNF-(alpha)-induced apoptosis at an early time point. However, cytokine- and Fas-induced signals to NF-(kappa)B are finally integrated by the (kappa)B kinase (IKK) complex, since (kappa)B[alpha] phosphorylation, NF-(kappa)B DNA binding activity, and IL-8 gene expression were strongly inhibited in HT-29 and HeLa cells overexpressing dominant-negative IKK[beta] (Ad5dnIKK[beta]). Our findings support the concept that cytokine signaling to NF-(kappa)B is redundant at the level of NIK. In addition, this study demonstrates for the first time the critical role of NIK and IKK[beta] in Fas-induced NF-(kappa)B signaling cascade.

Lysophosphatidic acid (LPA) is a mediator of multiple cellular responses. LPA mediates its effects predominantly through the G protein-coupled receptors, LPA1, LPA2, and LPA3. In the present work, we studied LPA2-mediated signaling using human colon cancer cell lines, which predominantly express LPA2. LPA2 activated Akt and Erk1/2 in response to LPA. LPA mediated Akt activation was inhibited by pertussis toxin (PTX), whereas Erk1/2 activation was completely inhibited by a blocker of phospholipase C(\(\beta\)), U73122. LPA also induced interleukin-8 (IL-8) synthesis in the colon cancer cells by primarily activating LPA2 receptor. We also found that LPA2 interacts with Na+/H+ exchanger regulatory factor 2 (NHERF2). Activation of Akt and Erk1/2 was significantly attenuated by silencing of NHERF2 expression by RNA interference, suggesting a pivotal role of NHERF2 in LPA2-mediated signaling. We also found that expression of LPA2 was elevated, whereas expression of LPA1 down-regulated in several types of cancers, including ovarian and colon cancer. We conclude that LPA2 is the major LPA receptor in colon cancer cells and cellular signals by LPA2 are largely mediated through its ability to interact with NHERF2.


Micro- and macroangiopathy are major causes of morbidity and mortality in patients with diabetes. Our aim was to characterize IGF-I receptor (IGF-IR) and insulin receptor (IR) in human micro- and macrovascular endothelial cells. Cultured human dermal microvascular endothelial cells (HMVEC) and human aortic endothelial cells (HAEC) were used. Gene expression was measured by quantitative real-time RT-PCR and receptor protein by ligand-binding assay. Phosphorylation of IGF-IR (\(\beta\))-subunit was analyzed by immunoprecipitation and Western blot. Glucose metabolism and DNA synthesis was assessed using [3H]glucose and [3H]thymidine incorporation, respectively. We detected gene expression of IGF-IR and IR in HAEC and HMVEC. IGF-IR gene expression was severalfold higher than that of IR. The specific binding of 125I-IGF-I was higher than that of 125I-insulin in HAEC and HMVEC. Insulin and the new, long-acting insulin analog glargine interacted with the IGF-IR with thousand- and hundred-fold less potency than IGF-I itself. Phosphorylation of the IGF-IR (\(\beta\))-subunit was shown in HAEC for IGF-I (10-8 M) and insulin (10-6 M) and in HMVEC for IGF-I and glargine (10-8 M, 10-6 M). IGF-I 10-7 M stimulated incorporation of [3H]thymidine into DNA, and 10-9-10-7 M also the incorporation of [3H]glucose in HMVEC, whereas glargine and insulin had no significant effects at 10-9-10-7 M. Human micro- and macrovascular endothelial cells express more IGF-IR than IR. IGF-I and high concentrations of glargine and insulin activates the IGF-IR. Glargine has a higher affinity than insulin for the IGF-IR but probably has no effect on DNA synthesis at concentrations reached in vivo.

Using in vitro and in vivo methods, we have demonstrated increased sensitivity of adrenocortical steroidogenesis to ACTH in Milan hypertensive (MHS) compared with normotensive (MNS) rats and have investigated whether this is caused by mutations of steroidogenic enzymes. Genes encoding aldosterone synthase (CYP11B2) and 11[beta]-hydroxylase (CYP11B1) in MHS and MNS have been cloned and sequenced. Nucleotide 752 (G) in exon 4 of MHS CYP11B2 differs from that of MNS (A); CYP11B1 sequences were identical. The nucleotide 752 mutation caused a Q251R substitution in the amino acid sequence of MHS CYP11B2. The phenotype of MHS CYP11B2 alleles, when expressed in COS-1 cells, differed from that of MNS alleles. The relative activities of the three reactions catalyzed by CYP11B2 (11[beta]-hydroxylation of deoxycorticosterone, 18-hydroxylation of corticosterone, and dehydrogenation of 18-hydroxycorticosterone) were estimated after incubation of transfected cells with [14C]deoxycorticosterone and analysis of radioactivity associated with deoxycorticosterone, corticosterone, 18-hydroxycorticosterone, and aldosterone. Both 11- and 18-hydroxylase activities were lower (19 and 12%, respectively; P < 0.01 and P < 0.05) in cells transfected with MHS compared with MNS alleles, whereas 18-oxidase activity was 42% higher (P < 0.01). To assess the significance of the CYP11B2 mutation in vivo, DNA from F2 hybrid MHS x MNS rats was genotyped. MHS alleles were associated with lower urine volumes in both sexes, lower ventricle weights in male rats, but no difference in systolic or diastolic blood pressures between the sexes. We conclude that a mutation in CYP11B2 may affect aldosterone secretion in MHS; however, under normal environmental circumstances, we were unable to demonstrate any influence of this mutation on blood pressure.


Type II 5'-iodothyronine deiodinase (D2), produces triiodothyronine (T3) and is stimulated by cold exposure via norepinephrine (NE) release in brown adipose tissue. Cultured rat brown adipocytes require T3 for the adrenergic stimulation of D2 activity. D2 mRNA expression in cultured brown adipocytes is undetectable with the use of basal conditions or NE without T3. Full D2 expression is achieved using NE + T3, especially after prolonged T3 exposure. [beta]3-Adrenergic agonists mimic the NE action, whereas cAMP analogs do not. Prolonged exposure to T3 alone increases D2 mRNA. High T3 doses (500 nM) inhibit the adrenergic stimulation of D2 activity while increasing D2 mRNA. The effects obtained with NE + T3 or T3 alone are suppressed by actinomycin, but not by cycloheximide, which leads to accumulation of short D2 mRNA transcripts. Prolonged or short exposure to T3 did not change D2 mRNA half-life, but T3 seemed to elongate it. In conclusion, T3 is an absolute requirement for the adrenergic stimulation of D2 mRNA in brown adipocytes. T3 upregulates D2 mRNA, an effect that might involve stimulation of factors required for transcription or for stabilization of D2 mRNA.


The transport mechanism mediating brain uptake of tumor necrosis factor (TNF)-[alpha] has been
studied. When 125I-labeled rat TNF-[alpha] was used in internal carotid artery perfusions in rats, the cytokine showed transcytosis through the blood-brain barrier in intact form (permeability-surface area product 0.34 \( \pm \) 0.13 \( \text{mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \)). Uptake was inhibited by low nanomolar concentrations of unlabeled rat TNF-[alpha]. Human TNF-[alpha], which does not interact with the p80 TNF receptor in rodents, showed no brain uptake. mRNA expression of both p60 and p80 receptors could be demonstrated in native brain microvessel preparations. These transcripts increased to 149\% (p60) and 127\% (p80) of control 4 h after a systemic immune stimulation (2 mg/kg bacterial endotoxin ip). Lipopolysaccharide treatment did not alter the rate of brain uptake of TNF-[alpha] measured between 4 and 24 h later. In conclusion, a receptor-mediated mechanism is responsible for the transcytosis of TNF-[alpha]. Saturable transport, requiring the p80 receptor, occurs at concentrations encountered under pathophysiological conditions and therefore constitutes a relevant mechanism of communication between the immune system and the brain.


http://ajpendo.physiology.org/cgi/content/abstract/284/5/E931

The incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gut hormones that act via the enteroinsular axis to potentiate insulin secretion from the pancreas in a glucose-dependent manner. Both GLP-1 receptor and GIP receptor knockout mice (GLP-1R[-]/[-] and GIPR[-]/[-], respectively) have been generated to investigate the physiological importance of this axis. Although reduced GIP action is a component of type 2 diabetes, GIPR-deficient mice exhibit only moderately impaired glucose tolerance. The present study was directed at investigating possible compensatory mechanisms that take place within the enteroinsular axis in the absence of GIP action. Although serum total GLP-1 levels in GIPR knockout mice were unaltered, insulin responses to GLP-1 from pancreas perfusions and static islet incubations were significantly greater (40-60\%) in GIPR[-]/[-] than in wild-type (GIPR+/+) mice. Furthermore, GLP-1-induced cAMP production was also elevated twofold in the islets of the knockout animals. Pancreatic insulin content and gene expression were reduced in GIPR[-]/[-] mice compared with GIPR+/+ mice. Paradoxically, immunocytochemical studies showed a significant increase in [beta]-cell area in the GIPR-null mice but with less intense staining for insulin. In conclusion, GIPR[-]/[-] mice exhibit altered islet structure and topography and increased islet sensitivity to GLP-1 despite a decrease in pancreatic insulin content and gene expression.

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http://ajpgi.physiology.org/cgi/content/abstract/282/3/G480

Regional differences in the ontogeny of mouse intestinal [alpha]-2,6-sialyltransferase activities ([alpha]-2,6-ST) and the influence of cortisone acetate (CA) on this expression were determined.
High ST activity and [alpha]-2,6-ST mRNA levels were detected in immature small and large intestine, with activity increasing distally from the duodenum. As the mice matured, ST activity (predominantly [alpha]-2,6-ST) in the small intestine decreased rapidly to adult levels by the fourth postnatal week. CA precociously accelerated this region-specific ontogenic decline. A similar decline of ST mRNA levels reflected ST activity in the small, but not the large, intestine. Small intestinal sialyl [alpha]-2,6-linked glycoconjugates displayed similar developmental and CA induced-precocious declines when probed using Sambucus nigra agglutinin (SNA) lectin. SNA labeling demonstrated age-dependent diminished sialyl [alpha]2,6 glycoconjugate expression in goblet cells in the small (but not large) intestine, but no such regional specificity was apparent in microvillus membrane. This suggests differential regulation of sialyl [alpha]-2,6 glycoconjugates in absorptive vs. globlet cells. These age-dependent and region-specific differences in sialyl [alpha]-2,6 glycoconjugates may be mediated in part by altered [alpha]-2,6-ST gene expression regulated by trophic factors such as glucocorticoids.


http://ajpgi.physiology.org/cgi/content/abstract/285/5/G919

Tumor necrosis factor-{alpha} (TNF-{alpha}) is a multifunctional cytokine involved in the expression of many genes integral to the inflammatory response. In addition, it activates both apoptotic and survival pathways, the latter being mediated through the activation of the transcription factor nuclear factor-{kappa}B (NF-{kappa}B). Protein kinase CK2, a serine-threonine kinase that is universally upregulated in human malignancies, may be involved at multiple levels in this process. However, its role in mediating a survival response within colon cancer cells remains incompletely understood. Here we report that inhibition of CK2 in HCT-116 and HT-29 cells with the use of two specific CK2 inhibitors, 5,6-dichloro-ribifuranosylbenzimidazole (DRB) and apigenin, effected a synergistic reduction in cell survival when used in conjunction with TNF-{alpha}. Furthermore, there was a demonstrable synergistic reduction in colony formation in soft agar with the use of the same combinations. Western blot analysis showed that poly-ADP ribose polymerase and procaspase-3 cleavage complemented the fluorescence-activated cell sorter analysis findings of significantly increased subdiploid DNA-containing cell populations using these conditions. Remarkably, these events occurred in the absence of any reduction in the expression of the Bcl-2 family members Bcl-2, Mcl-1, and Bcl-xL or any change in the proapoptotic molecules Bad or Bax. One-hybrid NF-{kappa}B promoter assays utilizing a Gal4-p65 transactivation domain construct revealed that the TNF-induced transactivation was inhibited by both DRB and apigenin. This was associated with a concomitant reduction in the expression of a recognized anti-apoptotic NF-{kappa}B target, manganese superoxide dismutase, demonstrated by Q-PCR. Our findings indicate a potentially novel strategy for the treatment of colon cancer, one that targets CK2 simultaneous with TNF-{alpha} administration.


http://ajpgi.physiology.org/cgi/content/abstract/284/6/G883

Previous studies suggest that ether-a-go-go related gene (ERG) KCNH2 potassium channels contribute to the control of motility patterns in the gastrointestinal tract of animal models. The present study examines whether these results can be translated into a role in human gastrointestinal muscles. Messages for two different variants of the KCNH2 gene were detected:
KCNH2 V1 human ERG (HERG) (28) and KCNH2 V2 (HERGUSO) (13). The amount of V2 message was greater than V1 in both human jejunum and brain. The base-pair sequence that gives rise to domains S3-S5 of the channel was identical to that previously published for human KCNH2 V1 and V2. KCNH2 protein was detected immunohistochemically in circular and longitudinal smooth muscle and enteric neurons but not in interstitial cells of Cajal. In the presence of TTX (10^{-6} M), atropine (10^{-6} M), and L-nitroarginine (10^{-4} M) human jejunal circular muscle strips contracted phasically (9 cycles/min) and generated slow waves with superimposed spikes. Low concentrations of the KCNH2 blockers E-4031 (10^{-8} M) and MK-499 (3 \times 10^{-8} M) increased phasic contractile amplitude and the number of spikes per slow wave. The highest concentration of E-4031 (10^{-6} M) produced a 10-20 mV depolarization, eliminated slow waves, and replaced phasic contractions with a small tonic contracture. E-4031 (10^{-6} M) did not affect [14C]ACh release from enteric neurons. We conclude that KCNH2 channels play a fundamental role in the control of motility patterns in human jejunum through their ability to modulate the electrical behavior of smooth muscle cells.


http://ajpgi.physiology.org/cgi/content/abstract/282/6/G1024

[gamma]/[delta] T cells might play an important role in autoimmune conditions like inflammatory bowel disease (IBD). In the present study, we characterized the T cell receptor (TCR)-[delta] repertoire by complementarity determining region 3 (CDR3) spectratyping in the inflamed and noninflamed mucosa and in the peripheral blood of subjects with Crohn's disease and ulcerative colitis. In contrast to previously published data about [alpha]/[beta] T cells, we rarely found oligoclonal expansions of [gamma]/[delta] T cells specific only for the inflamed mucosa. The same dominant [gamma]/[delta] T cell expansions were also present in the noninflamed colon. Furthermore, the peripheral [gamma]/[delta] TCR repertoire was oligoclonal but clearly distinct from that in the inflamed intestine. Thus our results do not support a role for antigen-specific [gamma]/[delta] T cells in IBD, and dominant [gamma]/[delta] T cells of the peripheral blood are not likely to be derived from the inflamed gut. However, in several patients, the TCR-[delta]-repertoire was highly diversified, whereas in others we observed a loss of dominant [gamma]/[delta] T cell clones when inflamed and noninflamed mucosa were compared. In conclusion, those changes indicate that [gamma]/[delta] T cells might play an important role in a subset of patients with IBD.


http://ajpgi.physiology.org/cgi/content/abstract/285/4/G714

Numerous genes expressed by intestinal epithelial cells are developmentally regulated, and the influence that adaptive (AI) and passive (PI) immunity have in controlling their expression has not been evaluated. In this study, we tested the hypothesis that both PI and AI influenced enterocyte gene expression by developing a breeding scheme that used T and B cell-deficient recombination-activating gene (RAG) mice. RNA was isolated from the liver and proximal/distal small intestine at various ages, and the steady-state levels of six different transcripts were evaluated by RNase protection assay. In wild-type (WT) pups, all transcripts (Fc receptor of the neonate (FcRn), polymeric IgA receptor (pIgR), GLUT5, lactase-phlorizin hydrolase (lactase), apical sodium-dependent bile acid transporter (ASBT), and Na+/glucose cotransporter (SGLT1)) studied were developmentally regulated at the time of weaning, and all transcripts except ASBT
had the highest levels of expression in the proximal small intestine. In WT suckling pups reared in the absence of PI, plgR mRNA levels were increased 100% during the early phase of development. In mice lacking AI, the expression of plgR and lactase were significantly attenuated, whereas FcRn and GLUT5 levels were higher compared with WT mice. Finally, in the absence of both passive and active immunity, expression levels of plgR and lactase were significantly lower than similarly aged WT mice. In summary, we report that the adaptive and passive immune status of mice influences steady-state mRNA levels of several important, developmentally regulated enterocyte genes during the suckling and weaning periods of life.


The nitric oxide (NO) synthase inhibitor N[omega]-nitro-L-arginine (L-NNA) inhibits heat stress (HS)-induced NO production and the inducible 70-kDa heat shock protein (HSP-70i) in many rodent organs. We used human intestinal epithelial T84 cells to characterize the inhibitory effect of L-NNA on HS-induced HSP-70i expression. Intracellular Ca2+ concentration ([Ca2+]i) was measured using fura-2, and protein kinase C (PKC), and PKA activities were determined. HS increased HSP-70i mRNA and protein in T84 cells exposed to 45[degrees]C for 10 min and allowed to recover for 6 h. L-NNA treatment for 1 h before HS inhibited the induction of HSP-70i mRNA and protein, with an IC50 of 0.0471 +/- 0.0007 {micro}M. Because the HS-induced increase in HSP-70i mRNA and protein is Ca2+ dependent, we measured [Ca2+]i after treating cells with L-NNA. L-NNA at 100 {micro}M significantly decreased resting [Ca2+]i. Likewise, treatment with 1 {micro}M GF-109203X or H-89 (inhibitors of PKC and PKA, respectively) for 30 min also significantly decreased [Ca2+]i and inhibited HS-induced increase in HSP-70i. GF-109203X- or H-89-treated cells failed to respond to L-NNA by further decreasing [Ca2+]i and HSP-70i. L-NNA effectively blocked heat shock factor-1 (HSF1) translocation from the cytosol to the nucleus, a process requiring PKC phosphorylation. These results suggest that L-NNA inhibits HSP-70i by reducing [Ca2+]i and decreasing PKC and PKA activity, thereby blocking HSF1 translocation from the cytosol to the nucleus.


Dietary lipid acutely upregulates apolipoprotein (apo) A-IV expression by sevenfold at the pretranslational level in neonatal swine jejunum. To determine the mechanism of this regulation, two-day-old female swine received intraduodenal infusions of low- and high-triacylglycerol (TG) isocaloric diets for 24 h. Nuclear runoff assay confirmed apo A-IV gene transcriptional regulation by the high-TG diet. Footprinting analysis using the swine apo A-IV proximal promoter sequence (+14 to [-]246 bp) demonstrated three regions protected by the low-TG extracts. Of these three motifs, only ACCTTC showed 100% homology to the human sequence and was further studied. EMSA was performed using probes containing wild-type (WT) and mutant (M) motifs. A shift was noted with the low-TG nuclear extracts with the WT probe but not with the M probe. Excess unlabeled free WT probe competed out the shift, whereas the M probe did not. No significant shift occurred with either probe using high-TG extracts. These results suggest that a repressor protein binds to the ACCTTC motif and becomes unbound during lipid absorption, allowing transcriptional activation of the apo A-IV gene in newborn swine small intestine.

http://ajpgi.physiology.org/cgi/content/abstract/287/5/G1062

The chemokine CCL28 is constitutively expressed by epithelial cells at several mucosal sites and is thought to function as a homeostatic chemoattractant of subpopulations of T cells and IgA B cells and to mediate antimicrobial activity. We report herein on the regulation of CCL28 in human colon epithelium by the proinflammatory cytokine IL-1, bacterial flagellin, and n-butyrate, a product of microbial metabolism. In vivo, CCL28 was markedly increased in the epithelium of pathologically inflamed compared with normal human colon. Human colon and small intestinal xenografts were used to model human intestinal epithelium in vivo. Xenografts constitutively expressed little, if any, CCL28 mRNA or protein. After stimulation with the proinflammatory cytokine IL-1, CCL28 mRNA and protein were significantly increased in the epithelium of colon but not small intestinal xenografts, although both upregulated the expression of another prototypic chemokine, CXCL8, in response to the identical stimulus. In studies of CCL28 regulation using human colon epithelial cell lines, proinflammatory stimuli, including IL-1, bacterial flagellin, and bacterial infection, significantly upregulated CCL28 mRNA expression and protein production. In addition, CCL28 mRNA expression and protein secretion by those cells were significantly increased by the short-chain fatty acid n-butyrate, and IL-1- or flagellin-stimulated upregulation of CCL28 by colon epithelial cells was synergistically increased by pretreatment of cells with n-butyrate. Consistent with its upregulated expression by proinflammatory stimuli, CCL28 mRNA expression was attenuated by pharmacological inhibitors of NF-κB activation. These findings indicate that CCL28 functions as an "inflammatory" chemokine in human colon epithelium and suggest the notion that CCL28 may act to counterregulate colonic inflammation.


http://ajpgi.physiology.org/cgi/content/abstract/285/1/G235

Numerous therapies used for inflammatory bowel disease (IBD) target the transcription factor NF-κB, which is involved in the production of cytokines and chemokines integral for inflammation. Here we show that curcumin, a component of the spice turmeric, is able to attenuate colitis in the dinitrobenzene sulfonic acid (DNB)-induced murine model of colitis. When given before the induction of colitis it reduced macroscopic damage scores and NF-κB activation. This was accompanied by a reduction in myeloperoxidase activity, and using semiquantitative RT-PCR, an attenuation of the DNB-induced message for IL-1β was detected. Western blotting analysis revealed that there was a reproducible DNB-induced activation of p38 MAPK detected in intestinal lysates by using a phosphospecific antibody. This signal was significantly attenuated by curcumin. Furthermore, we show that the immunohistochemical signal is dramatically attenuated at the level of the mucosa by curcumin. We conclude that the widely used food additive curcumin is able to attenuate experimental colitis through a mechanism correlated with the inhibition of the activation of NF-κB and effects a reduction in the activity of p38 MAPK. We propose that this agent may have therapeutic implications for human IBD.

The interleukin-2-deficient (IL-2-/-) mouse model of ulcerative colitis was used to test the hypothesis that colonic epithelial cells (CEC) directly respond to bacterial antigens and that alterations in Toll-like receptor (TLR)-mediated signaling may occur during the development of colitis. TLR expression and activation of TLR-mediated signaling pathways in primary CEC of healthy animals was compared with CEC in IL-2-/- mice during the development of colitis. In healthy animals, CEC expressed functional TLR, and in response to the TLR4 ligand LPS, proliferated and secreted the cytokines IL-6 and monocyte chemoattractant protein-1 (MCP-1). However, the TLR-responsiveness of CEC in IL-2-/- mice was different with decreased TLR4 responsiveness and augmented TLR2 responses that result in IL-6 and MCP-1 secretion. TLR signaling in CEC did not involve NF-κB (p65) activation with the inhibitory p50 form of NF-κB predominating in CEC in both the healthy and inflamed colon. Development of colitis was, however, associated with the activation of MAPK family members and upregulation of MyD88-independent signaling pathways characterized by increased caspase-1 activity and IL-18 production. These findings identify changes in TLR expression and signaling during the development of colitis that may contribute to changes in the host response to bacterial antigens seen in colitis.


Proliferation and carcinogenesis of the large intestinal epithelial cells (IEC) cells is significantly increased in transgenic mice that overexpress the precursor progastrin (PG) peptide. It is not known if the in vivo growth effects of PG on IEC cells are mediated directly or indirectly. Full-length recombinant human PG (rhPG1-80) was generated to examine possible direct effects of PG on IEC cells. Surprisingly, rhPG (0.1-1.0 nM) was more effective than the completely processed gastrin 17 (G17) peptide as a growth factor. Even though IEC cells did not express CCK1 and CCK2 receptors (-R), fluorescently labeled G17 and Gly-extended G17 (G-Gly) were specifically bound to the cells, suggesting the presence of binding proteins other than CCK1-R and CCK2-R on IEC cells. High-affinity (Kd = 0.5-1.0 nM) binding sites for 125I-rhPG were discovered on IEC cells that demonstrated relative binding affinity for gastrin-like peptides in the order PG [≥] COOH-terminally extended G17 [≥] G-Gly > G17 > *CCK-8 (* significant difference; P < 0.05). In conclusion, our studies demonstrate for the first time direct growth effects of the full-length precursor peptide on IEC cells in vitro that are apparently mediated by the high-affinity PG binding sites that were discovered on these cells.


Regulation of bilirubin glucuronide transporters during hyperbilirubinemia in hepatic and extrahepatic tissues is not completely clear. In the present study, we evaluated the regulation of the bilirubin glucuronide transporters, multidrug resistance-associated proteins (MRP)2 and 3, in rats with obstructive jaundice. Bile duct ligation (BDL) or sham operation was performed in Wistar rats. Liver and kidneys were removed 1, 3, and 5 days after BDL (n = 4, in each group). Serum
and urine were collected to measure bilirubin levels just before animal killing. MRP2 and MRP3 mRNA expressions were determined by real-time RT-PCR. Protein expression of MRP2 and MRP3 was determined by Western blotting. Renal MRP2 function was evaluated by para-aminohippurate (PAH) clearance. The effect of conjugated bilirubin, unconjugated bilirubin, human bile, and sulfate-conjugated bile acid on MRP2 gene expression was also evaluated in renal and hepatocyte cell lines. Serum bilirubin and urinary bilirubin excretion increased significantly after BDL. In the liver, the mRNA expression of MRP2 decreased 59, 86, and 82%, and its protein expression decreased 25, 74, and 93% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. In contrast, the liver expression of MRP3 mRNA increased 138, 2,137, and 3,295%, and its protein expression increased 560, 634, and 612% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. On the other hand, in the kidneys, the mRNA expression of MRP2 increased 162, 73, and 21%, and its protein expression increased 387, 558, and 472% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. PAH clearance was significantly increased after BDL. The mRNA expression of MRP2 increased in renal proximal tubular epithelial cells after treatment with conjugated bilirubin, sulfate-conjugated bile acid or human bile. Upregulation of MRP2 in the kidneys and MRP3 in the liver may be a compensatory mechanism to improve bilirubin clearance during obstructive jaundice.


http://ajpgi.physiology.org/cgi/content/abstract/282/1/G145

Endotoxemia causes an inflammatory response within the intestinal muscularis and gastrointestinal dysmotility. We hypothesize that the resident macrophage-derived chemokine monocyte chemoattractant protein-1 (MCP-1) plays a significant role in the recruitment of leukocytes into the lipopolysaccharide (LPS)-stimulated rat intestinal muscularis. MCP-1 mRNA expression was investigated by RT-PCR. Leukocyte extravasation and MCP-1 protein localization were determined by immunohistochemistry. Contractile activity was assessed by using a standard organ bath in rats that were treated with saline, recombinant MCP-1, LPS, LPS + nonspecific antibody, or LPS + MCP-1 antibody. Endotoxemia caused a significant 280-fold increase in MCP-1 mRNA expression in the muscularis, peaking at 3 h. MCP-1 protein was immunohistochemically located to muscularis macrophages. LPS application caused significant leukocyte recruitment into the muscularis and a 51% decrease in muscle contractility. MCP-1 antibody treatment significantly averted leukocyte recruitment and significantly prevented muscle dysfunction. These parameters were not significantly altered by the nonspecific antibody. Results show that resident muscularis macrophage-derived MCP-1 plays a major role in the recruitment of monocytes during endotoxemia, which then subsequently secrete kinetically active substances that cause ileus.


http://ajpgi.physiology.org/cgi/content/abstract/287/3/G555

Lactase-phlorizin hydrolase gene expression is spatially restricted along the anterior-posterior gut axis. Lactase gene transcription is maximal in the distal duodenum and jejunum in adult mammals and is barely detectable in the proximal duodenum. By contrast, pancreatic duodenal homeobox-1 (PDX-1) protein is expressed maximally in the proximal duodenum. This study aimed to determine the role of PDX-1 in regulating lactase gene promoter activity in intestinal epithelial cells. Caco-2 cells were cotransfected with lactase promoter-reporter constructs in the
presence of a PDX-1 expression vector and assayed for luciferase activity. PDX-1 cotransfection results in repression of lactase promoter activity. Sequence analysis of the lactase promoter revealed a putative PDX-1 DNA binding site in the proximal 100-bp lactase gene promoter. EMSAs demonstrated that PDX-1 can interact with the lactase promoter binding site but not with a site in which the core PDX-1 binding sequence TAAT is mutated. Site-directed mutagenesis of the PDX-1 core binding site in the lactase promoter-reporter construct suggests that PDX-1 can function independently of DNA binding to its consensus binding site. Stable overexpression of PDX-1 results in repression of the endogenous human lactase gene in differentiated Caco-2 cells. Given the contrasting spatial expression pattern, PDX-1 may function to specify the anterior boundary of lactase expression in the small intestine and is thus a candidate regulator of anterior spatial restriction in the gut.


http://ajpgi.physiology.org/cgi/content/abstract/282/1/G184

Cholestasis is associated with retention of bile acids and reduced expression of the Na+/taurocholate cotransporter (Ntcp), the major hepatocellular bile acid uptake system. This study aimed to determine whether downregulation of Ntcp in obstructive cholestasis 1) is a consequence of bile acid retention and 2) is mediated by induction of the transcriptional repressor short heterodimer partner 1 (SHP-1). To study the time course for the changes in serum bile acid levels as well as SHP-1 and Ntcp steady-state mRNA levels, mice were subjected to common bile duct ligation (CBDL) for 3, 6, 12, 24, 72, and 168 h and compared with sham-operated controls. Serum bile acid levels were determined by radioimmunoassay. SHP-1 and Ntcp steady-state mRNA expression were assessed by Northern blotting. In addition, Ntcp protein expression was studied by Western blotting and immunofluorescence microscopy. Increased SHP-1 mRNA expression paralleled elevations of serum bile acid levels and was followed by downregulation of Ntcp mRNA and protein expression in CBDL mice. Maximal SHP-1 mRNA expression reached a plateau phase after 6-h CBDL (12-fold; P < 0.001) and preceded the nadir of Ntcp mRNA levels (12%, P < 0.001) by 6 h. In conclusion, bile acid-induced expression of SHP-1 may, at least in part, mediate downregulation of Ntcp in CBDL mice. These findings support the concept that downregulation of Ntcp in cholestasis limits intracytoplasmatic accumulation of potentially toxic bile acids.

Am J Physiol Heart Circ Physiol (15)


http://ajpheart.physiology.org/cgi/content/abstract/00320.2004v1

Heart failure (HF) is characterized by marked prolongation of the action potential duration and a reduction in cellular repolarization reserve. These changes are caused, in large part, by HF-induced Potassium (K)-current down regulation. Molecular mechanisms underlying these changes remain uncertain. We determined whether down regulation of K currents in a canine
model of tachycardia-induced HF is caused by altered expression of underlying \{alpha\} and \{beta\} K channel subunits encoding these currents. K channel subunit expression was quantified in normal and failing dogs at the mRNA and protein levels in epicardial (EPI), midmyocardial (MID), and endocardial (ENDO) layers of the left ventricle. Results: Analysis of mRNA and protein levels of candidate genes encoding the transient-outward K-current (Ito) revealed marked reductions in cKv4.3 expression in HF in EPI (44% mRNA, 39% protein), MID (52% mRNA, 34% protein), and ENDO (49% mRNA, 73% protein) layers, a paradoxical enhancement (41% EPI, 97% MID, 113% ENDO) in cKv1.4 protein levels, without significant changes in cKChIP2 expression. Expression of cKir2.1, the gene underlying IK1, was unaffected by HF at mRNA and protein levels despite significant reduction in IK1, while remarkably canine-ERG, encoding IKr, exhibited increased protein expression. HF was not accompanied by significant changes in cKvLQT1 or cMinK mRNA and protein levels. Conclusions: These data indicate that: 1) Down-regulation of Ito in HF is associated with decreased cKv4.3, and not cKv1.4 or cKChIP2. 2) Alterations in IKr, IKs and IK1 in non-ischemic dilated cardiomyopathy are not caused by changes in either transcript or immunoreactive protein levels of relevant channel subunits, suggesting post-translational modification of these currents by HF.


http://ajpheart.physiology.org/cgi/content/abstract/286/4/H1354

We studied molecular and functional characteristics as well as hormonal regulation of the Na-K-2Cl cotransporter (NKCC) in the isolated rat heart and cardiomyocytes. NKCC activity was measured as bumetanide-sensitive 86Rb+ influx in isolated perfused rat hearts and isolated cardiomyocytes. Stimulation of \{alpha\}1-adrenoceptors (AR) by phenylephrine (30 \{micro\}M) increased 86Rb+ influx. The NKCC inhibitor bumetanide (50 \{micro\}M) reduced the response to phenylephrine by 45 +/- 13% (n = 12, P < 0.01). PD-98059 (10 \{micro\}M), an inhibitor of the activation of the mitogen-activated protein kinases extracellular signal-regulated protein kinase 1 and 2 (ERK1/2), reduced the total response to phenylephrine by 51 +/- 13% (n = 10, P < 0.01) and eliminated the bumetanide-sensitive component, indicating that \{alpha\}1-AR mediated stimulation of NKCC is dependent on activation of ERK1/2. Inhibitors of protein kinase C or phosphatidylinositol 3-kinase had no effect. The presence of NKCC mRNA and protein was demonstrated in isolated rat cardiomyocytes. Phosphorylation of NKCC after \{alpha\}1-AR stimulation was shown by immunoprecipitation of the phosphoprotein from 32P prelabeled cardiomyocytes. Increased phosphorylation of the NKCC protein was also abolished by PD-98059. We conclude that the NKCC is present in rat cardiomyocytes and that ion transport by the cotransporter is regulated by \{alpha\}1-AR stimulation through phosphorylation of this protein involving the ERK pathway.


http://ajpheart.physiology.org/cgi/content/abstract/284/5/H1778

Arteries remodel in response to environmental changes. We investigated whether mechanical strain modulates production of matrix metalloproteinase (MMP)-2 and -9 by cultured vascular smooth muscle cells (SMC). MMP-2 and MMP-9 expression were tested using human saphenous vein SMC cultured on silicone membranes at rest or subjected to physiological levels (5%) of stationary or cyclical (1 Hz) uniaxial strain. Compared with control, stationary strain significantly increased MMP-2 mRNA levels at all time points, whereas cyclic strain decreased it after 48 h.
Both secreted and cell-associated pro-MMP-2 levels were increased by stationary strain at all times (P < 0.01), whereas cyclic strain decreased secreted levels after 48 h (P < 0.02). MMP-9 mRNA levels and pro-MMP-9 protein were increased after 48 h of stationary stretch (P < 0.01) compared with both no strain and cyclic strain. Our study indicates that vascular SMC show a selective response to different types of strain. We suggest that local increases in stationary mechanical strain resulting from stenting, hypertension, or atherosclerosis may lead to enhanced matrix degradation by SMC.


http://ajpheart.physiology.org/cgi/content/abstract/01220.2004v1

Despite the important roles played by ventricular fibroblasts and myofibroblasts in formation and maintenance of the extracellular matrix, neither the ionic basis for membrane potential, nor the effect of modulating membrane potential on function, have been analyzed in detail. In this study, whole-cell patch clamp experiments were done using ventricular fibroblasts and myofibroblasts. Time- and voltage-dependent outward K+ currents were recorded at depolarized potentials, and an inwardly rectifying K+ (Kir) current was recorded near the resting membrane potential (RMP) and at more hyperpolarized potentials. The apparent reversal potential of Kir currents shifted to more positive potentials as external K+ concentration ([K+]o) was raised and this Kir current was blocked by 100 - 300 micromolar Ba2+. RT-PCR measurements showed that mRNA for Kir2.1 was expressed. Accordingly, we conclude that Kir current is the primary determinant of RMP in both fibroblasts and myofibroblasts. Changes in [K+]o influenced fibroblast membrane potential as well as proliferation and contractile functions. Recordings made with a voltage-sensitive dye, DiBAC3(4), showed that 1.5 mM [K+]o resulted in a hyperpolarization, while 20 mM [K+]o produced a depolarization. Low [K+]o (1.5 mM) enhanced myofibroblast number relative to control (5.4 mM [K+]o). In contrast, 20 mM [K+]o resulted in a significant reduction in myofibroblast number. In separate assays, 20 mM [K+]o significantly enhanced contraction of collagen I gels seeded with myofibroblasts, compared to control mechanical activity in 5.4 mM [K+]o. In combination these results show that ventricular fibroblasts and myofibroblasts express a variety of K+ channel alpha subunits and demonstrate that Kir current can modulate RMP and alter essential physiological functions.


http://ajpheart.physiology.org/cgi/content/abstract/286/6/H2042

Platelet-derived growth factor (PDGF)-BB, a potent mitogen for mesenchymal cells, also downregulates expression of multiple smooth muscle (SM) cell (SMC)-specific markers. However, there is conflicting evidence whether PDGF-BB represses SMC marker expression at a transcriptional or posttranscriptional level, and little is known regarding the mechanisms responsible for these effects. Results of the present studies provide clear evidence that PDGF-BB treatment strongly repressed SM {alpha}-actin, SM myosin heavy chain (MHC), and SM22{alpha} promoters in SMCs. Of major significance for resolving previous controversies in the field, we found PDGF-BB-induced repression of SMC marker gene promoters in subconfluent, but not postconfluent, cultures. Treatment of postconfluent SMCs with a tyrosine phosphatase inhibitor restored PDGF-BB-induced repression, whereas treatment of subconfluent SMCs with a tyrosine kinase blocker abolished PDGF-BB-induced repression, suggesting that a tyrosine
phosphorylation event mediates cell density-dependent effects. On the basis of previous observations that Ets-1 transcription factor is upregulated within phenotypically modulated neointimal SMCs, we tested whether Ets-1 would repress SMC marker expression. Consistent with this hypothesis, results of cotransfection experiments indicated that Ets-1 overexpression reduced transcriptional activity of SMC marker promoter constructs in SMCs, whereas it increased activity of SM (alpha)-actin promoter in endothelial cells. PDGF-BB treatment increased expression of Ets-1 in cultured SMCs, and SM (alpha)-actin mRNA expression was reduced in multiple independent clones of SMCs stably transfected with an Ets-1-overexpressing construct. Taken together, results of these experiments provide novel insights regarding possible mechanisms whereby PDGF-BB and Ets-1 may contribute to SMC phenotypic switching associated with vascular injury.


http://ajpheart.physiology.org/cgi/content/abstract/284/6/H2255

We developed an RT-PCR assay to study both the time course and the mechanism for the triiodothyronine (T3)-induced transcription of the [alpha]- and [beta]-myosin heavy chain (MHC) genes in vivo on the basis of the quantity of specific heterogeneous nuclear RNA (hnRNA). The temporal relationship of changes in transcriptional activity to the amount of [alpha]-MHC mRNA and the coordinated regulation of transcription of more than one gene in response to T3 are demonstrated here for the first time. Quantitation of [alpha]-MHC hnRNA demonstrated that T3 induced [alpha]-MHC transcription in hypothyroid rats within 30 min of a single injection of T3 (0.5 μg/100 g body wt). Maximal transcription rates (135% +/- 15.8 of euthyroid values) occurred 6 h after injection and subsequently declined in parallel with serum T3 levels. The transcription of [beta]-MHC was reduced to 86% of peak hypothyroid levels 6 h after a single T3 injection and reached a nadir of 59% of hypothyroid levels at 36 h. Analysis of the time course of T3-mediated induction of [alpha]-MHC hnRNA and repression of [beta]-MHC hnRNA indicates that separate molecular mechanisms are involved in the coordinated regulation of these genes.


http://ajpheart.physiology.org/cgi/content/abstract/285/4/H1759

Angiogenesis, the growth of new blood vessels from existing ones, occurs in the skeletal muscle as an adaptive response to exercise that satisfies the increased requirement of this tissue for oxygen delivery and metabolic processes. Of the factors that have been identified to regulate this process, the endothelial cell mitogen vascular endothelial growth factor (VEGF) has been proposed to play a key role. The aim of this study was to measure the skeletal muscle VEGF mRNA content and arteriovenous protein balance across the working leg in response to a single bout of prolonged, submaximal exercise. Seven physically active males completed 3 h of two-legged kicking ergometry. Muscle biopsies were collected from the vastus lateralis muscle from both working legs, and blood samples were collected from one femoral artery and femoral vein before, during, and in recovery from exercise. We show that the exercise stimulus elicited a decrease in VEGF protein arteriovenous balance across the exercising leg (P = 0.007), and a ninefold elevation in skeletal muscle VEGF mRNA expression (P < 0.001). The changes in VEGF protein balance and mRNA content were most pronounced 1 h after the cessation of exercise. In conclusion, these findings demonstrate that submaximal exercise, suitable for humans with low CV fitness, induces a decrease in VEGF arteriovenous balance that is likely to be of clinical
significance in promoting angiogenic effects.


http://ajpheart.physiology.org/cgi/content/abstract/282/2/H784

Mitogenic effects of the extracellular nucleotides ATP and UTP are mediated by P2Y1, P2Y2, and P2Y4 receptors. However, it has not been possible to examine the highly expressed UDP-sensitive P2Y6 receptor because of the lack of stable, selective agonists. In rat aorta smooth muscle cells (vascular smooth muscle cells; VSMC), UDP and UTP stimulated 3H-labeled thymidine incorporation with similar pEC50 values (5.96 and 5.69). Addition of hexokinase did not reduce the mitogenic effect of UDP. In cells transfected with P2Y receptors the stable pyrimidine agonist uridine 5'-O-(2-thiodiphosphate) (UDP[beta]S) was specific for P2Y6 with no effect on P2Y1, P2Y2, or P2Y4 receptors. UDP[beta]S stimulated [3H]thymidine and [3H]leucine incorporation and increased cell number in VSMC. Flow cytometry demonstrated that UDP stimulated cell cycle progression to both the S and G2 phases. The intracellular signal pathways were dependent on phospholipase C, possibly protein kinase C-[delta], and a tyrosine kinase pathway but independent of Gi proteins, eicosanoids, and protein kinase A. The half-life of P2Y6 receptor mRNA was <1 h by competitive RT-PCR. The mitogen-activated protein kinase kinase inhibitor PD-098059 significantly suppressed, whereas ATP and interleukin-1[beta] upregulated, expression of P2Y6 receptor mRNA. The results demonstrate that UDP stimulates mitogenesis through activation of P2Y6 receptors and that the receptor is regulated by factors important in the development of vascular disease.


http://ajpheart.physiology.org/cgi/content/abstract/286/6/H2257

T-type Ca2+ channels are implicated in cardiac automaticity, cell growth, and cardiovascular remodeling. Two voltage-gated Ca2+ subtypes (Cav3.1 and Cav3.2) have been cloned for the pore-forming {alpha}1-subunit of the T-type Ca2+ channel in cardiac muscle, but their differential roles remain to be clarified. The aim of this study was to elucidate the relative contribution of the two subtypes in the normal development of mouse hearts. A whole cell patch clamp was used to record ionic currents from ventricular myocytes isolated from mice of early (E9.5) and late embryonic days (E18) and from adult 10-wk-old mice. Large T-type Ca2+ current (ICa,T) was observed at both E9.5 and E18, displaying similar voltage-dependence and kinetics of activation and inactivation. The current was inhibited by Ni2+ at relatively low concentrations (IC50 26-31 {micro}M). ICa,T was undetectable in adult myocytes. Quantitative PCR analysis revealed that Cav3.2 mRNA is the predominant subtype encoding T-type Ca2+ channels at both E9.5 and E18. Cav3.1 mRNA increased from E9.5 to E18, but remained low compared with Cav3.2 mRNA during the whole embryonic period. In the adulthood, in contrast, Cav3.1 mRNA is greater than Cav3.2 mRNA. These results indicate that Cav3.2 underlies the functional T-type Ca2+ channels in the embryonic murine heart, and there is a subtype switching of transcripts from Cav3.2 to Cav3.1 in the perinatal period.

Pourdjabbar, A., T. G. Parker, et al. (2005). "Effects of pre-, peri-, and postmyocardial infarction treatment with losartan in rats: effect of dose on survival, ventricular arrhythmias, function, and remodeling."
Angiotensin receptor blockers (ARBs) reduce adverse left ventricular (LV) remodeling and improve LV function and survival when started postmyocardial infarction (MI). ARBs also reduce ventricular arrhythmias during ischemia-reperfusion injury when started pre-MI. No information exists regarding their efficacy and safety when started pre-MI and continued peri- and post-MI. We evaluated whether the ARB losartan improves the outcome when started pre-MI and continued peri- and post-MI. Male Wistar rats (n = 502) were treated for 7 days pre-MI with losartan at a high dose (30 mg·kg⁻¹·day⁻¹), progressively increasing dose (3 mg·kg⁻¹·day⁻¹ increased to 10 mg·kg⁻¹·day⁻¹ 10 days and 30 mg·kg⁻¹·day⁻¹ 20 days post-MI), or no treatment. Ambulatory systolic blood pressure and Holter monitoring were performed for 24 h post-MI. Echocardiography was done 30 days post-MI, and LV remodeling, cardiac hemodynamics, and fetal gene expression were assessed 38 days post-MI. High-dose losartan reduced 24-h post-MI survival compared with the progressive dose and control (21.9% vs. 36.6% and 38.1%, P = 0.033 and P = 0.009, respectively). This was associated with greater hypotension in the high dose and no change in ventricular arrhythmias in all groups. In 24-h post-MI survivors, the progressive dose group had reduced mortality from 24 h to 38 days (8.5% vs. 28.6% for control vs. 38.9% for high dose, P = 0.032 and P = 0.01, respectively). Survivors of both losartan groups demonstrated improved LV remodeling, cardiac hemodynamics, preserved GLUT-4, and reduced cardiac fetal gene expression. Pretreatment with ARBs does not reduce 24-h post-MI ventricular arrhythmias or survival, and high doses increase mortality by causing excessive hypotension. In 24-h post-MI survivors, progressively increasing doses of losartan have multiple beneficial effects, including improved survival.


We tested the hypothesis that TRPC3, a member of the canonical transient receptor potential (TRP) family of channels, mediates agonist-induced depolarization of arterial smooth muscle cells (SMCs). In support of this hypothesis, we observed that suppression of arterial SMC TRPC3 expression with antisense oligodeoxynucleotides significantly decreased the depolarization and constriction of intact cerebral arteries in response to UTP. In contrast, depolarization and contraction of SMCs induced by increased intravascular pressure, i.e., myogenic responses, were not altered by TRPC3 suppression. Interestingly, UTP-evoked responses were not affected by suppression of a related TRP channel, TRPC6, which was previously found to be involved in myogenic depolarization and vasoconstriction. In patch-clamp experiments, UTP activated a whole cell current that was greatly reduced or absent in TRPC3 antisense-treated SMCs. These results indicate that TRPC3 mediates UTP-induced depolarization of arterial SMCs and that TRPC3 and TRPC6 may be differentially regulated by receptor activation and mechanical stimulation, respectively.

The goal of this study was to determine the role of estrogen receptor subtypes in the development of pressure overload hypertrophy in mice. Epidemiological studies have suggested gender differences in the development of hypertrophy and heart disease, but the mechanism and the role of estrogen receptor subtypes are not established. We performed transverse aortic constriction (TAC) and sham operations in male and female wild-type (WT) mice and mice lacking functional estrogen receptor-\(\alpha\) \{\(\alpha\)-estrogen receptor knockout (\(\alpha\)-ERKO)\} and mice lacking estrogen receptor-\(\beta\) (\(\beta\)-ERKO). Body, heart, and lung weights were measured 2 wk postsurgery. WT male mice subjected to TAC showed a 64% increase in the heart weight-to-body weight ratio (HW/BW) compared with sham, and WT males have increased lung weight at 2 wk. WT female mice subjected to TAC showed a 31% increase in HW/BW compared with sham, which was significantly less than their male counterparts and with no evidence of heart failure. \(\alpha\)-ERKO females developed HW/BW nearly identical to that seen in WT littermate females in response to TAC, indicating that estrogen receptor-\(\alpha\) is not essential for the attenuation of hypertrophy observed in WT females. In contrast, \(\beta\)-ERKO females responded to TAC with a significantly greater increase in HW/BW than WT littermate females. \(\beta\)-ERKO females have lower expression of lipoprotein lipase at baseline than WT or \(\alpha\)-ERKO females. These data suggest an important role for estrogen receptor-\(\beta\) in attenuating the hypertrophic response to pressure overload in females.


http://ajpheart.physiology.org/cgi/content/abstract/284/1/H268

Dilated cardiomyopathy, a disease of unknown etiology and pathogenesis, is associated with heart failure and compensatory hypertrophy. Although cell and animal models suggest a role for altered gene expression in the transition to heart failure, there is a paucity of data derived from the study of human heart tissue. In this study, we used DNA microarray profiling to investigate changes in the expression of genes involved in apoptosis that occur in human idiopathic dilated cardiomyopathic hearts that had progressed to heart failure. We observed altered gene expression consistent with a proapoptotic shift in the TNF-\(\alpha\) signaling pathway. Specifically, we found decreased expression of TNF-\(\alpha\)- and NF-\(\kappa\)B-induced antiapoptotic genes such as growth arrest and DNA damage-inducible (GADD)45[\(\beta\)], Flice inhibitory protein (FLIP), and TNF-induced protein 3 (A20). Consistent with a role for apoptosis in heart failure, we also observed a significant decrease in phosphorylation of BAD at Ser-112. This study identifies several pathways that are altered in human heart failure and provides new targets for therapy.


http://ajpheart.physiology.org/cgi/content/abstract/287/3/H1296

Ghrelin, a newly identified endogenous ligand for growth hormone secretagogue receptor 1a (GHSR-1a, i.e., ghrelin receptor), was recently demonstrated to be a potent vasoactive peptide. Although sepsis is characterized by an early, hyperdynamic phase, it remains unknown whether ghrelin or GHSR-1a plays a role in the cardiovascular response to sepsis. To determine this, polymicrobial sepsis was induced by cecal ligation and puncture in male adult rats. At 5 h (i.e., early sepsis) or 20 h (i.e., late sepsis) after cecal ligation and puncture, blood and tissue samples were collected. Ghrelin levels and ghrelin and GHSR-1a mRNA expression were assessed by RIA and RT-PCR, respectively. In addition, GHSR-1a protein levels in aorta, heart, and small intestine were determined by Western blotting. The vascular response to ghrelin was determined by using an isolated gut preparation. A primary rat aortic smooth muscle cell culture was used to
determine the effects of LPS on GHSR-1a expression. The results indicate that although ghrelin levels decreased at early and late sepsis, its receptor was markedly elevated in early sepsis. Moreover, ghrelin-induced relaxation in resistance blood vessels of the isolated small intestine increased significantly during early sepsis but was not altered in late sepsis. Furthermore, GHSR-1a expression in smooth muscle cells was significantly increased at mRNA and protein levels with stimulation by LPS at 10 ng/ml. These results demonstrate that GHSR-1a expression is upregulated and vascular sensitivity to ghrelin stimulation is increased in the hyperdynamic phase of sepsis.


http://ajpheart.physiology.org/cgi/content/abstract/285/6/H2770

T-type Ca2+ channels may play a role in cardiac development. We studied the developmental regulation of the T-type currents (ICa,T) in cardiomyocytes (CMs) derived from mouse embryonic stem cells (ESCs). ICa,T was studied in isolated CMs by whole cell patch clamp. Subsequently, CMs were identified by the myosin light chain 2v-driven green fluorescent protein expression, and laser capture microdissection was used to isolate total RNA from groups of cells at various developmental time points. ICa,T showed characteristics of Cav3.1, such as resistance to Ni2+ block, and a transient increase during development, correlating with measures of spontaneous electrical activity. Real-time RT-PCR showed that Cav3.1 mRNA abundance correlated (r2 = 0.81) with ICa,T. The mRNA copy number was low at 7+4 days (2 copies/cell), increased significantly by 7+10 days (27/cell; P < 0.01), peaked at 7+16 days (174/cell), and declined significantly at 7+27 days (25/cell). These data suggest that ICa,T is developmentally regulated at the level of mRNA abundance and that this regulation parallels measures of pacemaker activity, suggesting that ICa,T might play a role in the spontaneous contractions during CM development.

Am J Physiol Lung Cell Mol Physiol  13


http://ajplung.physiology.org/cgi/content/abstract/286/1/L112

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract disease in infants and children worldwide. Intranasal infection of BALB/c mice with RSV strain A2, but not ultraviolet-inactivated RSV, for 2 or 4 days reduced basal alveolar fluid clearance (AFC), a seminal function of bronchoalveolar epithelium, and caused loss of AFC sensitivity to amiloride inhibition. Reduced AFC was temporally associated with increased lung water content but was not a consequence of increased epithelial permeability or cell death. Reduced AFC was also not due to decreased transcription of epithelial Na+ channel subunit genes in lung tissue. RSV-mediated inhibition of AFC 2 days after infection was rapidly prevented by addition to the instillate of P2Y receptor antagonists (suramin and XAMR-0721) or enzymes that degrade UTP, but not those that degrade ATP. After UTP degradation, AFC returned to control levels but was no longer
sensitive to amiloride. UTP at nanomolar concentrations recapitulated the AFC inhibitory effect of RSV in normal mice and mice infected with RSV for 6 days, indicating that normalization of AFC at this time point is a consequence of cessation of UTP release, rather than P2Y receptor desensitization. We conclude that RSV infection of the bronchoalveolar epithelium results in reduced AFC as a consequence of autocrine feedback inhibition mediated by UTP. These studies are the first to demonstrate AFC inhibition by an important pulmonary viral pathogen. Reduced AFC may result in formation of an increased volume of fluid mucus, airway congestion, and rhinorrhea, all features of severe RSV disease.


http://ajplung.physiology.org/cgi/content/abstract/287/5/L962

Transient receptor potential (TRP) cation channels are a critical pathway for Ca2+ entry during pulmonary artery (PA) smooth muscle contraction. However, whether canonical TRP (TRPC) subunits and which TRP channel isoforms are involved in store depletion-induced pulmonary vasoconstriction in vivo remain unclear. This study was designed to test whether overexpression of the human TRPC1 gene (hTRPC1) in rat PA enhances pulmonary vasoconstriction due to store depletion-mediated Ca2+ influx. The hTRPC1 was infected into rat PA rings with an adenoviral vector. RT-PCR and Western blot analyses confirmed the mRNA and protein expression of hTRPC1 in the arterial rings. The amplitude of active tension induced by 40 mM K+ (40K) in PA rings infected with an empty adenoviral vector (647 {±7/} 88 mg/mg) was similar to that in PA rings infected with hTRPC1 (703 {±7/} 123 mg/mg, P = 0.3). However, the active tension due to capacitative Ca2+ entry (CCE) induced by cyclopiazonic acid was significantly enhanced in PA rings overexpressing hTRPC1 (91 {±7/} 13% of 40K-induced contraction) compared with rings infected with an empty adenoviral vector (61 {±7/} 14%, P < 0.001). Endothelial expression of hTRPC1 was not involved since the CCE-induced vasoconstriction was also enhanced in endothelium-denuded PA rings infected with the adenoviral vector carrying hTRPC1. These observations demonstrate that hTRPC1 is an important Ca2+-permeable channel that mediates pulmonary vasoconstriction when PA smooth muscle cell intracellular Ca2+ stores are depleted.


http://ajplung.physiology.org/cgi/content/abstract/285/6/L1354

To test the hypothesis that chronic intrauterine pulmonary hypertension (PHTN) compromises pulmonary artery (PA) smooth muscle cell (SMC) O2 sensing, fluorescence microscopy was used to study the effect of an acute increase in PO2 on the cytosolic Ca2+ concentration ([Ca2+]i) of chronically hypoxic subconfluent monolayers of PA SMC in primary culture. PA SMCs were derived from fetal lambs with PHTN due to intrauterine ligation of the ductus arteriosus. Acute normoxia decreased [Ca2+]i in control but not PHTN PA SMC. In control PA SMC, [Ca2+]i increased after Ca2+-sensitive (KCa) and voltage-sensitive (Kv) K+ channel blockade and decreased after diltiazem treatment. In PHTN PA SMC, KCa blockade had no effect, whereas Kv blockade and diltiazem increased [Ca2+]i. Inhibition of sarcoplasmic reticulum Ca2+ ATPase activity caused a greater increase in [Ca2+]i in controls compared with PHTN PA SMC. Conversely, ryanodine caused a greater increase of [Ca2+]i in PHTN compared with control PA SMC. KCa channel mRNA is decreased and Kv channel mRNA is unchanged in PHTN PA SMC compared with controls. We conclude that PHTN compromises PA SMC O2 sensing, alters
intracellular Ca2+ homeostasis, and changes the predominant ion channel that determines basal [Ca2+]i from KCa to Kv.


http://ajplung.physiology.org/cgi/content/abstract/00200.2004v1

Expression of cell adhesion molecule (CAM) in endothelial cells upon activation by human immunodeficiency virus (HIV) infection is associated with the development of atherosclerotic vasculopathy. We postulated that induction of vascular cell adhesion molecule-1 (VCAM-1) by HIV-1 tat protein in endothelial cells might represent an early event that could culminate in inflammatory cell recruitment and vascular injury. We determined the role of HIV-1 tat protein in VCAM-1 expression in human pulmonary artery endothelial cells (HPAECS). HIV-1 tat protein treatment significantly increased cell-surface expression of VCAM-1 in HPAECs. Consistently, mRNA expression of VCAM-1 was also increased by HIV-1 tat protein as measured by RT-PCR. HIV-1 tat protein-induced VCAM-1 expression was abolished by the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) and the p38 MAPK inhibitor SB203580. Furthermore, HIV-1 tat protein enhanced DNA binding activity of NF-κB, facilitated nuclear translocation of NF-κB subunit p65, and increased production of reactive oxygen species (ROS). Similarly to VCAM-1 expression, HIV-1 tat protein-induced NF-κB activation and ROS generation were abrogated by PDTC and SB203580. These data indicate that HIV-1 tat protein is able to induce VCAM-1 expression in HPAECs, which may represent a pivotal early molecular event in HIV-induced vascular/pulmonary injury. These data also suggest that molecular mechanism underlying the HIV-1 tat protein-induced VCAM-1 expression may involve ROS generation, p38 MAPK activation and NF-κB translocation, which are the characteristics of pulmonary endothelial cell activation.


http://ajplung.physiology.org/cgi/content/abstract/287/6/L1333

Surfactant protein D (SP-D) is a member of the collectin subfamily of C-type lectins, pattern recognition proteins participating in the innate immune response. Gene-targeted mice deficient in SP-D develop abnormalities in surfactant homeostasis, hyperplasia of alveolar epithelial type II cells, and emphysema-like pathology. Granulocyte/macrophage colony-stimulating factor (GM-CSF) is required for terminal differentiation and subsequent activation of alveolar macrophages, including the expression of matrix metalloproteinases and reactive oxygen species, factors thought to contribute to lung remodeling. Type II cells also express the GM-CSF receptor. Thus we hypothesized GM-CSF might mediate some or all of the cellular and structural abnormalities in the lungs of SP-D-deficient mice. To test this, SP-D (D-G+) and GM-CSF (D+G-) single knockout mice as well as double knockout mice deficient for both SP-D and GM-CSF (D-G-) were analyzed by design-based stereology. Compared with wild type, D-G+ as well as D+G- mice showed decreased alveolar numbers, increased alveolar sizes, and decreased alveolar epithelial surface areas. These emphysema-like changes were present to a greater extent in D-G- mice. D-G+ mice developed type II cell hyperplasia and hypertrophy with increased intracellular surfactant pools, whereas D+G- mice had smaller type II cells with decreased intracellular surfactant pools. In contrast to the emphysematous changes, the type II cell alterations were mostly corrected in D-G- mice. These results indicate that GM-CSF-dependent macrophage activity is not necessary for emphysema development in SP-D-deficient mice, but that type II cell metabolism and proliferation
are, either directly or indirectly, regulated by GM-CSF in this model.


http://ajplung.physiology.org/cgi/content/abstract/287/2/L318

Oei, Erwin, Thomas Kalb, Prarthana Beuria, Matthieu Allez, Atsushi Nakazawa, Miyuki Azuma, Michael Timony, Zanetta Stuart, Houchu Chen, and Kirk Sperber. Accessory cell function of airway epithelial cells. We previously demonstrated that airway epithelial cells (AECs) have many features of accessory cells, including expression of class II molecules CD80 and CD86 and functional Fc(\gamma) receptors. We have extended these studies to show that freshly isolated AECs have mRNA for cathepsins S, V, and H [proteases important in antigen (Ag) presentation], invariant chain, human leukocyte antigen (HLA)-DM-[alpha] and HLA-DM-[beta], and CLIP, an invariant chain breakdown product. A physiologically relevant Ag, ragweed, was colocalized with HLA-DR in AECs, and its uptake was increased by granulocyte-macrophage colony-stimulating factor and IFN-\{gamma\} treatments, which had no effect on CD80 and CD86 expression. We demonstrate the presence of other costimulatory molecules, including B7h and B7-H1, on AECs and the increased expression of B7-H1 on AECs after treatment with granulocyte-macrophage colony-stimulating factor and IFN-{gamma}. Finally, we compared T cell proliferation after allostimulation with AECs and dendritic cells (DCs). The precursor frequency of peripheral blood T cells responding to AECs was 0.264\% compared with 0.55\% for DCs. DCs stimulated CD45RO+, CD45RA+, CCR7+ and CCR7-CD4+, and CD8+ T cells, whereas AECs stimulated only CD45RO+, CD45RA-, CCR7-, CD4+, and CD8+ T cells. There was no difference in cytokine production, type of memory T cells stimulated (effector vs. long-term memory), or apoptosis by T cells cocultured with AECs and DCs. The localization of AECs exposed to the external environment may make them important in the regulation of local immune responses.


http://ajplung.physiology.org/cgi/content/abstract/282/3/L546

Mycobacterium tuberculosis (Mtb) infection induces the expression of matrix metalloproteinase-9 (MMP-9) in mouse lungs. In cultured human monocytic cells, Mtb bacilli and the cell wall glycolipid lipoarabinomannan (LAM) stimulate high levels of MMP-9 activity. Here, we explore the cellular mechanisms involved in the induction of MMP-9 by Mtb. We show that infection of THP-1 cells with Mtb caused a fivefold increase in MMP-9 mRNA that was associated with increased MMP-9 activity. MMP-9 induction was dependent on microtubule polymerization and protein kinase activation and was associated with increased DNA binding by the transcription factor activator protein-1 (AP-1), which appeared to be important for MMP-9 expression. We then explored the surface molecules potentially involved in Mtb induction of MMP-9, focusing on ligands of the mannose and [beta]-glucan receptors. MMP-9 activity was induced by the mannose receptor ligands mannan, zymosan, and LAM, whereas the [beta]-glucan receptor ligand laminarin was not effective. The most active inducers of MMP-9 activity were the particulate ligand zymosan and LAM. Pretreatment of cells with an anti-mannose receptor monoclonal antibody, but not anti-complement receptor 3, decreased the induction of MMP-9 activity by Mtb bacilli. Together, these results suggest that MMP-9 induction by Mtb occurs by receptor-mediated signaling mechanisms involving the binding of mannosylated ligands to mannose receptors, the modulation by cytoskeletal elements such as microtubules, the activation of protein kinases, and transcriptional activation by AP-1.
Staphylococcus aureus [alpha]-toxin is a pore-forming bacterial exotoxin that has been implicated as a significant virulence factor in human staphylococcal diseases. In primary cultures of rat pneumocyte type II cells and the human A549 alveolar epithelial cell line, purified [alpha]-toxin provoked rapid-onset phosphatidylinositol (PtdIns) hydrolysis as well as liberation of nitric oxide and the prostanoids PGE2, PGI2, and thromboxane A2. In addition, sustained upregulation of proinflammatory interleukin (IL)-8 mRNA expression and protein secretion occurred. "Priming" with low-dose IL-1[beta] markedly enhanced the IL-8 response to [alpha]-toxin, which was then accompanied by IL-6 appearance. The cytokine response was blocked by the intracellular Ca2+-chelating reagent 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, the protein kinase C inhibitor bis-indolyl maleimide I, as well as two independent inhibitors of nuclear factor-[kappa]B activation, pyrrolidine dithiocarbamate and caffeic acid phenethyl ester. We conclude that alveolar epithelial cells are highly reactive target cells of staphylococcal [alpha]-toxin. [alpha]-Toxin pore-associated transmembrane Ca2+ flux and PtdIns hydrolysis-related signaling with downstream activation of protein kinase C and nuclear translocation of nuclear factor-[kappa]B are suggested to represent important underlying mechanisms. Such reactivity of the alveolar epithelial cells may be relevant for pathogenic sequelae in staphylococcal lung disease.

In this study, we examined the sequential expression of several matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors as well as the presence of apoptosis in a model of pulmonary fibrosis induced in rats with paraquat and hyperoxia. Animals showing neither clinical nor morphological changes with this double aggression were classified as "resistant". Rats were killed at 1, 2, 3, and 6 wk, and lungs were used for collagen content, gene expression by real-time PCR, gelatinolytic activity by zymography, apoptosis by in situ DNA fragmentation, and protein localization by immunohistochemistry. Our results showed a significant decrease of collagenases MMP-8 and MMP-13, with an increase of TIMP-1 and transforming growth factor-(beta). Immunoreactive TIMP-1 was increased in experimental rats and primarily localized in alveolar macrophages. Expression of gelatinases MMP-2 and MMP-9 mRNAs was not affected, but lung zymography revealed an increase in progelatinase B, progelatinase A, and its active form. Epithelial apoptosis was evident from the first week, whereas at later periods, interstitial cell apoptosis was also noticed. Resistant animals behave as controls. These findings suggest that an imbalance between collagenases and TIMPs, excessive gelatinolytic activity, and epithelial apoptosis participate in the fibrotic response in this experimental model.
Surfactant protein B (SP-B) is a developmentally and hormonally regulated lung protein that is required for normal surfactant function. We generated transgenic mice carrying the human SP-B promoter ([-]1,039/+431 bp) linked to chloramphenicol acetyltransferase (CAT). CAT activity was high in lung and immunoreactive protein localized to alveolar type II and bronchiolar epithelial cells. In addition, thyroid, trachea, and intestine demonstrated CAT activity, and each of these tissues also expressed low levels of SP-B mRNA. Developmental expression of CAT activity and SP-B mRNA in fetal lung were similar and both increased during explant culture. SP-B mRNA but not CAT activity decreased during culture of adult lung, and both were reduced by transforming growth factor (TGF)-[beta]1. Treatment of adult mice with intratracheal bleomycin caused similar time-dependent decreases in lung SP-B mRNA and CAT activity. These findings indicate that the human SP-B promoter fragment directs tissue- and lung cell-specific transgene expression and contains cis-acting elements involved in regulated expression during development, fetal lung explant culture, and responsiveness to TGF-[beta] and bleomycin-induced lung injury.


http://ajplung.physiology.org/cgi/content/abstract/283/1/L144

Pulmonary vascular medial hypertrophy due to proliferation of pulmonary artery smooth muscle cells (PASMC) greatly contributes to the increased pulmonary vascular resistance in pulmonary hypertension patients. A rise in cytosolic free Ca2+ concentration ([Ca2+]cyt) is an important stimulus for cell growth in PASMC. Resting [Ca2+]cyt, intracellularly stored [Ca2+], capacitative Ca2+ entry (CCE), and store-operated Ca2+ currents (ISOC) are greater in proliferating human PASMC than in growth-arrested cells. Expression of TRP1, a transient receptor potential gene proposed to encode the channels responsible for CCE and ISOC, was also upregulated in proliferating PASMC. Our aim was to determine if inhibition of endogenous TRP1 gene expression affects ISOC and CCE and regulates cell proliferation in human PASMC. Cells were treated with an antisense oligonucleotide (AS, for 24 h) specifically designed to cleave TRP1 mRNA and then returned to normal growth medium for 40 h before the experiments. Then, mRNA and protein expression of TRP1 was downregulated, and amplitudes of ISOC and CCE elicited by passive depletion of Ca2+ from the sarcoplasmic reticulum using cyclopiazonic acid were significantly reduced in the AS-treated PASMC compared with control. Furthermore, the rate of cell growth was decreased by 50% in AS-treated PASMC. These results indicate that TRP1 may encode a store-operated Ca2+ channel that plays a critical role in PASMC proliferation by regulating CCE and intracellular [Ca2+]cyt.


http://ajplung.physiology.org/cgi/content/abstract/286/4/L777

Retinoic acid (RA) is known to accelerate wound healing and induce cell differentiation. All-trans RA (ATRA) exerts its effect by binding retinoic acid receptors, which are members of the nuclear receptor family. We investigated whether RA can alter expression of eotaxin, a potent eosinophil chemoattractant that is regulated by the transcription factors signal transducer and activator of transcription 6 (STAT6) and NF-(kappa)B. We examined the effects of RA on eotaxin expression in a human bronchial epithelial cell line BEAS-2B. ATRA and its stereodimer 9-cis retinoic acid (9-cis RA) inhibited IL-4-induced release of eotaxin at 10-6 M by 78.0 and 52.0%, respectively (P < 0.05). ATRA and 9-cis RA also significantly inhibited IL-4-induced eotaxin mRNA expression at 10-6 M by 52.3 and 53.5%, respectively (P < 0.05). In contrast, neither ATRA nor 9-cis RA had
any effects on TNF-\(\alpha\)-induced eotaxin production. In transfection studies using eotaxin promoter luciferase plasmids, the inhibitory effect of ATRA on IL-4-induced eotaxin production was confirmed at the transcriptional level. Interestingly, ATRA had no effects on IL-4-induced tyrosine phosphorylation, nuclear translocation, or DNA binding activity of STAT6. Activating protein-1 was not involved in ATRA-mediated transrepression of eotaxin with IL-4 stimulation. The mechanism of the inhibitory effect of ATRA on IL-4-induced eotaxin production in human bronchial epithelial cells has not been elucidated but does not appear to be due to an effect on STAT6 activation. These findings raise the possibility that RA may reduce eosinophilic airway inflammation, one of the prominent pathological features of allergic diseases such as bronchial asthma.


http://ajplung.physiology.org/cgi/content/abstract/287/6/L1323

Connective tissue growth factor (CTGF), a potent profibrotic mediator, acts downstream and in concert with transforming growth factor (TGF)-\(\beta\) to drive fibrogenesis. Significant upregulation of CTGF has been reported in fibrogenic diseases, including idiopathic pulmonary fibrosis (IPF), and is partly responsible for associated excessive fibroblast proliferation and extracellular matrix deposition, but no effective therapy exists for averting such fibrogenic events. Simvastatin has reported putative antifibrotic actions in renal fibroblasts; this study explores such actions on human IPF-derived and normal lung fibroblasts and examines associated driving mechanisms. Simvastatin reduces basal CTGF gene and protein expression in all fibroblast lines, overriding TGF-\(\beta\) induction through inhibition of the cholesterol synthesis pathway. Signaling pathways driving simvastatin's effects on CTGF/TGF-\(\beta\) interaction were evaluated using transient reporter transfection of a CTGF promoter construct. Inhibition of CTGF promoter activity by simvastatin was most marked at 10 \(\mu\)M concentration, reducing activity by 76.2 and 51.8% over TGF-\(\beta\)-stimulated cultures in IPF and normal fibroblasts, respectively. We also show that geranylgeranylpyrophosphate (GGPP), but not farnesylpyrophosphate, induces CTGF promoter activity following simvastatin inhibition by 55.3 and 31.1% over GGPP-negative cultures in IMR90 and IPF-derived fibroblasts, respectively, implicating small GTPase Rho involvement rather than Ras in these effects. Indeed, the specific Rho inhibitor C3 exotoxin significantly (\(P < 0.05\)) suppressed TGF-\(\beta\)-induced CTGF promoter activity in transfected lung fibroblasts, a finding further supported by transfection of dominant-negative and constitutively active RhoA constructs, thus demonstrating that simvastatin through a Rho signaling mechanism in lung fibroblasts can modulate CTGF expression and interaction with TGF-\(\beta\).

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http://ajpregu.physiology.org/cgi/content/abstract/287/4/R943
In obesity-related hypertension, activation of the renin-angiotensin system (RAS) has been reported despite marked fluid volume expansion. Adipose tissue expresses components of the RAS and is markedly expanded in obesity. This study evaluated changes in components of the adipose and systemic RAS in diet-induced obese hypertensive rats. RAS was quantified in adipose tissue and compared with primary sources for the circulating RAS. Male Sprague-Dawley rats were fed either a low-fat (LF; 11% kcal as fat) or moderately high-fat (32% kcal as fat) diet for 11 wk. After 8 wk, rats fed the moderately high-fat diet segregated into obesity-prone (OP) and obesity-resistant (OR) groups based on their body weight gain (body weight: OR, 566 +/- 10; OP, 702 +/- 20 g; P < 0.05). Mean arterial blood pressure was increased in OP rats (LF: 97 +/- 2; OR: 105 +/- 1 mmHg; P < 0.05). Quantification of mRNA expression by real-time PCR demonstrated a selective increase (2-fold) in angiotensinogen gene expression in retroperitoneal adipose tissue from OP vs. OR and LF rats. Similarly, plasma angiotensinogen concentration was increased in OP rats (LF: 390 +/- 48; OR: 355 +/- 24; OP: 530 +/- 22 ng/ml; P < 0.05). In contrast, other components of the RAS were not altered in OP rats. Marked increases in the plasma concentrations of angiotensin peptides were observed in OP rats (angiotensin II: LF: 95 +/- 31; OR: 59 +/- 20; OP: 295 +/- 118 pg/ml; P < 0.05). These results demonstrate increased activity of the adipose and systemic RAS in obesity-related hypertension.


http://ajpregu.physiology.org/cgi/content/abstract/287/1/R69

Many mammals, nearing the end of life, spontaneously decrease their food intake and body weight, a stage we refer to as senescence. The spontaneous decrease in food intake and body weight is associated with attenuated responses to intracerebroventricular injections of neuropeptide Y (NPY) compared with old presenescent or with young adult rats. In the present study, we tested the hypothesis that this blunted responsiveness involves the number and expression of hypothalamic paraventricular nucleus (PVN) Y1 and/or Y5 NPY receptors, both of which are thought to mediate NPY-induced food intake. We found no significant difference in mRNA levels, via quantitative PCR, for Y1 and Y5 receptors in the PVN of senescent vs. presenescent rats. In contrast, immunohistochemistry indicated that the number of PVN neurons staining for Y1 receptor protein was greater in presenescent compared with senescent rats. We conclude that a decreased expression and number of Y1 or Y5 receptors in the PVN cannot explain the attenuated responsiveness of the senescent rats to exogenous NPY.


http://ajpregu.physiology.org/cgi/content/abstract/288/5/R1316

Burn injury has been shown to impair gut transit, but the exact mechanism remains unknown. The present study investigated whether nitric oxide synthase (NOS) and cyclooxygenase (COX) mediated changes in burn-induced colonic transit. After rats underwent 30% total body surface area burn injury, they were injected with S-methylisothiourea (SMT, selective inducible NOS inhibitor), 7-nitronidazole (7-NI, selective neuronal NOS inhibitor), and nimesulide (NIM, selective COX-2 inhibitor), respectively. The protein and mRNA of NOS and COX-2 were measured by Western blot analysis and real-time RT-RCR, and localization of NOS and COX-2 protein was determined by immunohistochemistry. Our results showed that colonic transit assessed by the geometric center was delayed from 3.47 +/- 0.28 in controls to 2.21 +/- 0.18 after burn (P <
SMT and NIM significantly improved colonic transit in burned rats but had no effect in sham-operated rats. 7-Ni failed to modify delayed transit in burned rats but significantly delayed colonic transit in sham-operated rats. Both protein and mRNA of inducible NOS and COX-2 increased significantly but not neuronal NOS in burned rats. Inducible NOS protein expression was noted not only in epithelial cells but also in neurons of the myenteric ganglia in burned rats. These findings suggest that nitric oxide (NO) produced by neuronal NOS plays an important role in mediating colonic transit under the physiological condition. NO produced by inducible NOS and prostaglandins synthesized by COX-2 are both involved in the pathogenesis of delayed colonic transit after burn injury. Inducible NOS expression in neurons of the myenteric ganglia may contribute to dysmotility with burn injury.


http://ajpregu.physiology.org/cgi/content/abstract/287/2/R397

The present study investigated the effect of an acute exercise bout on the mRNA response of vascular endothelial growth factor (VEGF) splice variants in untrained and trained human skeletal muscle. Seven habitually active young men performed one-legged knee-extensor exercise training at an intensity corresponding to ~70% of the maximal workload in an incremental test five times/week for 4 wk. Biopsies were obtained from the vastus lateralis muscle of the trained and untrained leg 40 h after the last training session. The subjects then performed 3 h of two-legged knee-extensor exercise, and biopsies were obtained from both legs after 0, 2, 6, and 24 h of recovery. Real-time PCR was used to examine the expression of VEGF mRNA containing exon 1 and 2 (all VEGF isoforms), exon 6 or exon 7, and VEGF165 mRNA. Acute exercise induced an increase (P < 0.05) in total VEGF mRNA levels as well as VEGF165 and VEGF splice variants containing exon 7 at 0, 2, and 6 h of recovery. The increase in VEGF mRNA was higher in the untrained than in the trained leg (P < 0.05). The results suggest that in human skeletal muscle, acute exercise increases total VEGF mRNA, an increase that appears to be explained mainly by an increase in VEGF165 mRNA. Furthermore, 4 wk of training attenuated the exercise-induced response in skeletal muscle VEGF165 mRNA.


http://ajpregu.physiology.org/cgi/content/abstract/283/6/R1450

Milk-borne insulin-like growth factors (IGFs) enhance nutrient absorption in the immature intestine, which is characterized by low levels of glucose oxidation. We therefore hypothesized that feeding a rat milk substitute (RMS) devoid of growth factors to rat pups would lower serum glucose levels relative to dam-fed control rats and that supplementation of RMS with physiological doses of either IGF-I or IGF-II would normalize serum glucose levels via increased jejunal glucose transporter 2 (GLUT2) and high-affinity Na+-glucose cotransporter (SGLT1) expression. We found lower serum glucose concentrations in RMS-fed pups; in contrast, serum glucose levels in the IGF-supplemented pups were similar to those of dam-fed controls. RT-PCR and laser scanning confocal microscopy similarly demonstrated that IGF supplementation increased expression of jejunal glucose transporters. Further experiments demonstrated that IGF supplementation altered mRNA levels of key mitochondrial enzymes without altering jejunal lactase activity. We conclude that IGF-I and IGF-II supplementation increases serum glucose levels in the immature rat pup fed artificial formula and alters gene expression of the jejunal
Bacterial lipopolysaccharide (LPS) induces fever that is mediated by pyrogenic cytokines such as interleukin (IL)-1[beta]. We hypothesized that the anti-inflammatory cytokine IL-10 modulates the febrile response to LPS by suppressing the production of pyrogenic cytokines. In rats, intravenous but not intracerebroventricular infusion of IL-10 was found to attenuate fever induced by peripheral administration of LPS (10 {micro}g/kg iv). IL-10 also suppressed LPS-induced IL-1[beta] production in peripheral tissues and in the brain stem. In contrast, central administration of IL-10 attenuated the febrile response to central LPS (60 ng/rat icv) and decreased IL-1[beta] production in the hypothalamus and brain stem but not in peripheral tissues and plasma. Furthermore, intravenous LPS upregulated expression of IL-10 receptor (IL-10R1) mRNA in the liver, whereas intracerebroventricular LPS enhanced IL-10R1 mRNA in the hypothalamus. We conclude that IL-10 modulates the febrile response by acting in the periphery or in the brain dependent on the primary site of inflammation and that its mechanism of action most likely involves inhibition of local IL-1[beta] production.


http://ajpregu.physiology.org/cgi/content/abstract/283/2/R496

Exposure to chronic hypoxia induces erythropoietin (EPO) production to facilitate oxygen delivery to hypoxic tissues. Previous studies from our laboratory found that ovariectomy (OVX) exacerbates the polycythemic response to hypoxia and treatment with 17[beta]-estradiol (E2-[beta]) inhibits this effect. We hypothesized that E2-[beta] decreases EPO gene expression during hypoxia. Because E2-[beta] can induce nitric oxide (NO) production and NO can attenuate EPO synthesis, we further hypothesized that E2-[beta] inhibition of EPO gene expression is mediated by NO. These hypotheses were tested in OVX catheterized rats treated with E2-[beta] (20 {micro}g/day) or vehicle for 14 days and exposed to 8 or 12 h of hypoxia (12% O2) or normoxia. We found that E2-[beta] treatment significantly decreased EPO synthesis and gene expression during hypoxia. E2-[beta] treatment did not induce endothelial NO synthase (eNOS) expression in the kidney but potentiated hypoxia-induced increases in plasma nitrates. We conclude that E2-[beta] decreases hypoxic induction of EPO. However, this effect does not appear to be related to changes in renal eNOS expression.


http://ajpregu.physiology.org/cgi/content/abstract/00771.2004v1

The present study tested the hypothesis that exercise with a large compared with a small active muscle mass results in a higher contraction induced increase in Na+, K+ -ATPase mRNA
expression, due to greater hormonal responses. Furthermore, the relative abundance of Na+, K+ -ATPase subunits \{alpha\}1, \{alpha\}2, \{alpha\}3, \{alpha\}4, \{beta\}1, \{beta\}2 and \{beta\}3 mRNA in human skeletal muscle was investigated. On two occasions, eight subjects performed one-legged knee-extension exercise (L) or combined one-legged knee-extension and bi-lateral arm cranking (AL) for 5.00, 4.25, 3.50, 2.75 and 2.00 min separated by 3 min of rest. Leg exercise power output was the same in AL and L (57\(+/-\)5 vs. 59\(+/-\)5 W), but heart rate (HR) at the end of each exercise interval was higher in AL compared with L (91\(+/-\)3\% vs. 61\(+/-\)12\% of maximal HR, \(P<0.001\)). One minute after exercise, arm venous blood lactate was higher (\(P<0.001\)) in AL than in L (11.4\(+/-\)4.2 vs. 4.2\(+/-\)2.2 mM). A higher (\(P<0.05\)) level of blood epinephrine (141\%) and nor-epinephrine (380\%) was evident three minutes after exercise in AL compared with L. Nevertheless, none of the exercise induced increases (\(P<0.001\)) in \{alpha\}1, \{alpha\}2, \{beta\}1 and \{beta\}3 mRNA expression levels were higher in AL compared with L. The most abundant Na+, K+ -ATPase subunit at the mRNA level was \{beta\}1, which was 3.4 times more expressed than \{alpha\}2 \(P<0.001\). Expression of \{alpha\}1, \{beta\}2 and \{beta\}3 was less than 5\% of the \{alpha\}2 expression, and no reliable detection of \{alpha\}3 and \{alpha\}4 was possible. In conclusion, activation of additional muscle mass does not result in a higher exercise induced increase in Na+, K+ -ATPase subunit specific mRNA.


http://ajpregu.physiology.org/cgi/content/abstract/288/2/R482

The rev-erbA\{alpha\} orphan protein belongs to the steroid nuclear receptor superfamily. No ligand has been identified for this protein, and little is known of its function in development or physiology. In this study, we focus on 1) the distribution of the rev-erbA\{alpha\} protein in adult fast- and slow-twitch skeletal muscles and muscle fibers and 2) how the rev-erbA\{alpha\} protein influences myosin heavy chain (MyHC) isoform expression in mice heterozygous (+/-) and homozygous (-/-) for a rev-erbA\{alpha\} protein null allele. In the fast-twitch extensor digitorum longus muscle, rev-erbA\{alpha\} protein expression was linked to muscle fiber type; however, MyHC isoform expression did not differ between wild-type, +/-, or -/- mice. In the slow-twitch soleus muscle, the link between rev-erbA\{alpha\} protein and MyHC isoform expression was more complex than in the extensor digitorum longus. Here, a significantly higher relative amount of the \{beta\}/slow (type I) MyHC isoform was observed in both rev-erbA\{alpha\} -/- and +/- mice vs. that shown in wild-type controls. A role for the ratio of thyroid hormone receptor proteins \{alpha\}1 to \{alpha\}2 in modulating MyHC isoform expression can be ruled out because no differences were seen in MyHC isoform expression between thyroid hormone receptor \{alpha\}2-deficient mice (heterozygous and homozygous) and wild-type mice. Therefore, our data are compatible with the rev-erbA\{alpha\} protein playing an important role in the regulation of skeletal muscle MyHC isoform expression.


http://ajpregu.physiology.org/cgi/content/abstract/282/4/R1164

The present study was designed to examine whether changes in Ca2+ release by inositol-1,4,5-trisphosphate (IP3) in 8-, 15-, and 30-day-old rat skeletal muscles could be associated with the expression of IP3 receptors. Experiments were conducted in slow-twitch muscle in which both IP3-induced Ca2+ release and IP3-receptor (IP3R) expression have been shown to be larger.
than in fast-twitch muscle. In saponin-skinned fibers, IP3 induced transient contractile responses in which the amplitude was dependent on the Ca2+-loading period with the maximal IP3 contracture being at 20 min of loading. The IP3 tension decreased during postnatal development, was partially inhibited by ryanodine (100 {micro}M), and was blocked by heparin (20-400 {micro}g/ml). Amplification of the DNA sequence encoding for IP3R isoforms (using the RT-PCR technique) showed that in slow-twitch muscle, the type 2 isoform is mainly expressed, and its level decreases during postnatal development in parallel with changes in IP3 responses in immature fibers. IP3-induced Ca2+ release would then have greater participation in excitation-contraction coupling in developing fibers than in mature muscle.


http://ajpregu.physiology.org/cgi/content/abstract/284/6/R1454

Previous reports implicate the orexins in eating and body weight regulation. This study investigated possible functional relationships between hypothalamic orexins and circulating hormones or metabolites. In situ hybridization and quantitative PCR were used to examine orexin expression in the perifornical hypothalamus (PF) of rats and mice on diets varying in fat content and with differential propensity toward obesity. The results showed that orexin gene expression was stimulated by a high-fat diet in close association with elevated triglyceride levels, suggesting a functional relationship between these measures. Results obtained in obesity-prone rats and mice revealed a similar increase in orexin in close relation to triglycerides. A direct test of this orexin-triglyceride link was performed with Intralipid, which increased PF orexin expression along with circulating triglycerides. Whereas PF galanin is similarly stimulated by dietary fat, double-labeling immunofluorescence studies showed that orexin and galanin neurons are anatomically distinct. This evidence suggests that the orexins, like galanin, are "fat-responsive" peptides that respond to circulating lipids.


http://ajpregu.physiology.org/cgi/content/abstract/283/5/R1198

Animals reared at 18{degrees}C exhibit enhanced innervation of brown adipose tissue (BAT) and greater cold tolerance as adults, yet gain more weight when fed an enriched diet compared with rats reared at 30{degrees}C. To explore this paradox, sympathoadrenal activity was examined using techniques of [3H]norepinephrine ([3H]NE) turnover and urinary catecholamine excretion in male and female rats reared until 2 mo of age at 18 or 30{degrees}C. Gene expression in BAT was also analyzed for several sympathetically related proteins. Although [3H]NE turnover in heart did not differ between groups, [3H]NE turnover in BAT was consistently elevated in the 18{degrees}C-reared animals, even 2 mo after removal from the cool environment. Gene expression for uncoupling proteins 1 and 3, GLUT-4, leptin, and the [alpha]1A-adrenergic receptor was more abundant in BAT and the increase in epinephrine excretion with fasting suppressed in 18{degrees}C-reared animals. These studies demonstrate that obesity consequent to exposure to 18{degrees}C in early life occurs despite tonic elevation of sympathetic input to BAT. Diminished adrenal epinephrine responsiveness to fasting may play a contributory role.
Mutations that disrupt a PY motif in epithelial Na⁺ channel (ENaC) subunits increase surface expression of Na⁺ channels in the collecting duct, resulting in greater Na⁺ reabsorption. Recently, Nedd4 and Nedd4-2 have been identified as ubiquitin ligases that can interact with ENaC via its PY motifs to regulate channel activity. To further understand the role of human Nedd4-2 (hNedd4-2), we cloned its cDNAs and determined its genomic organization using a bioinformatic approach. The gene is present as a single copy, spans at least 400 kb, and contains >40 exons. Multiple 5'-exons were identified by 5'-rapid amplification of cDNA ends, and tissue-specific expression of these transcripts was noted by RT-PCR and RNase protection assay. Alternate polyadenylation signal sequences led to varying lengths of the 3'-untranslated region. Alternate splicing events within internal exons were also noted. Open reading frame analysis indicates that hNedd4-2 encode multiple protein variants with and without a C2 domain, and with a variable number of WW domains. Coexpression, in Fischer rat thyroid epithelia, of ENaC and Nedd4-2 cDNAs leads to a significant reduction in amiloride-sensitive currents, confirming a role in Na⁺ transport regulation. In vitro binding studies demonstrated that individual PY motifs of {alpha}-, {beta}-, and {gamma}-ENaC have strong affinity for WW domains 3 and 4 but not 1 and 2. These studies indicate that alternate transcripts of Nedd4-2 may interact with ENaC differently. Understanding the function of variant proteins will increase our knowledge of the role of hNedd4-2 in the regulation of ENaC and define protein domains important for Nedd4-2 function.


Evaluation of thick ascending limb (TAL) function has been hindered by the limited ability to selectively examine the function of this nephron segment in vivo. To address this, a Cre/loxP strategy was employed whereby the Tamm-Horsfall (THP) promoter was used to drive Cre recombinase expression in transgenic mice. The THP gene was cloned from a mouse genomic library, and 3.7 kb of the mouse THP 5'-flanking region containing the first noncoding exon of the THP gene were inserted upstream of an epitope-tagged Cre recombinase (THP-CreTag). THP-CreTag transgenic mice were bred with ROSA26-enhanced yellow fluorescent protein (eYFP) mice (contain a loxP-flanked “STOP” sequence 5’ to eYFP), and doubly heterozygous offspring were analyzed. THP and eYFP were expressed in an identical pattern with predominant localization to the renal outer medulla without expression in nonrenal tissues. eYFP did not colocalize with thiazide-sensitive cotransporter (distal tubule) or neuronal nitric oxide synthase (macula densa) expression. THP mRNA expression was detected only in kidney, whereas CreTag mRNA was also present in testes. These data indicate that THP-CreTag transgenic mice can be used for TAL-specific gene recombination in the kidney.
Dengue serotype 2 (DEN-2) viruses with the potential to cause dengue hemorrhagic fever have been shown to belong to the Southeast (SE) Asian genotype. These viruses appear to be rapidly displacing the American genotype of DEN-2 in the Western Hemisphere. To determine whether distinct genotypes of DEN-2 virus are better adapted to mosquito transmission, we classified 15 viral strains of DEN-2 phylogenetically and compared their ability to infect and disseminate in different populations of Aedes aegypti mosquitoes. Envelope gene nucleotide sequence analysis confirmed that six strains belonged to the American genotype and nine strains were of the SE Asian genotype. The overall rate of disseminated infection in mosquitoes from Texas was 27% for the SE Asian genotype versus 9% for the American genotype. This pattern of infection was similar in another population of mosquitoes sampled from southern Mexico (30% versus 13%). Together, these findings suggest that Ae. aegypti tends to be more susceptible to infection by DEN-2 viruses of the SE Asian genotype than to those of the American genotype, and this may have epidemiologic implications.

It is still unclear to what extent myocarditis-associated, chronic Chagas' heart disease is due to persisting Trypanosoma cruzi. In the present study, we have analyzed tissue samples from the hearts of three patients with this disease. In situ hybridization provided little evidence for the presence of intact T. cruzi, even at sites of strong inflammation. Nevertheless, micromanipulation techniques detected remnants of both T. cruzi kinetoplast DNA and nuclear DNA. Trypanosoma cruzi DNA was also detected in single macrophages dissected directly from frozen heart tissue sections. Thus, this analysis demonstrates that T. cruzi kinetoplast DNA and nuclear DNA are widely dispersed in the heart tissue, although in low amounts. Since we rarely detected intact T. cruzi parasites during the chronic phase of Chagas' heart disease, we can exclude heart tissue as a major parasite reservoir.

We present a large case-control candidate gene study of leprosy susceptibility. Thirty-eight polymorphic sites from 13 genes were investigated for their role in susceptibility to leprosy by
comparing 270 cases with 452 controls in Karonga district, northern Malawi. Homozygotes for a silent T[→]C change in codon 352 of the vitamin D receptor gene appeared to be at high risk (odds ratio [OR] = 4.3, 95% confidence interval [CI] = 1.6-11.4, P = 0.004), while homozygotes for the McCoy b blood group defining variant K1590E in exon 29 of the complement receptor 1 (formerly CD35) gene appeared to be protected (OR = 0.3, 95% CI = 0.1-0.8, P = 0.02). Borderline evidence for association with leprosy susceptibility was found for seven polymorphic sites in an additional six genes. Some of these apparent associations may be false-positive results from multiple comparisons, and several associations suggested by studies in other populations were not replicated here. These data provide evidence of inter-population heterogeneity in leprosy susceptibility.


http://www.ajtmh.org/cgi/content/abstract/69/6_suppl/39

As part of a larger vaccine study, peripheral blood mononuclear cells (PBMC) were collected from volunteers for analysis of vaccine-induced T cell responses. The PBMC were re-stimulated in vitro with live dengue virus and assayed for Th1 or Th2 memory cell responses. Re-stimulated PBMC from the volunteers predominantly secreted interferon-(gamma). Little interleukin-4 (IL-4) or IL-10 secretion was detected, indicating a Th1 type of T cell response. The interferon-(gamma) response was primarily serotype-specific with some serotype cross-reactivity. T cell depletion studies showed that the interferon-(gamma) was being secreted by CD4+ T lymphocytes and/or by cells other than CD8+ T lymphocytes that were being stimulated by the CD4+ T lymphocytes. CD3+ or CD8+ T cell depletion showed that granzyme B mRNA expression correlated with the presence of CD4+ T lymphocytes. However, depletion of CD4+ T cells after four days of stimulation indicated that the granzyme B mRNA was produced by cells in culture other than lymphocytes. In summary, an antigen-specific Th1 type T cell response was seen as a response to vaccination using live attenuated dengue virus.


http://www.ajtmh.org/cgi/content/abstract/69/5/506

The objective of the study was to determine if the presence or absence of virulence factor-positive and -negative enteroaggregative Escherichia coli (EAEC) determined the occurrence of illness or sub-clinical EAEC infection in travelers from the United States to Mexico. Sixty-five newly arrived college students from the United States submitted weekly stool samples for a four-week period of time. Among EAEC-infected subjects, diarrhea occurred in those with a defined virulence factor with the following frequency: aggA, 5 of 15 (33%); aggR, 3 of 11 (27%); aafA, 3 of 8 (38%); and aspU, 1 of 6 (17%). Twenty-two of 31 students (71%) had two or more EAEC infections. After the initial EAEC infection, only 4 (11%) of 31 students had a subsequent symptomatic EAEC infection. Our study suggests that clinical illness by EAEC is not explained by presence of a defined EAEC virulence factors, and we provide suggestive evidence that EAEC infection protects against future symptomatic infection.
Two specific and sensitive polymerase chain reaction (PCR) assays were developed to detect and quantitate Orientia tsutsugamushi, the agent of scrub typhus, using a portion of the 47-kD outer membrane protein antigen/ high temperature requirement A gene as the target. A selected 47-kD protein gene primer pair amplified a 118-basepair fragment from all 26 strains of O. tsutsugamushi evaluated, but it did not produce amplicons when 17 Rickettsia and 18 less-related bacterial nucleic acid extracts were tested. Similar agent specificity for the real-time PCR assay, which used the same primers and a 31-basepair fluorescent probe, was demonstrated. This sensitive and quantitative assay determination of the content of O. tsutsugamushi nucleic acid used a plasmid containing the entire 47-kD gene from the Kato strain as a standard. Enumeration of the copies of O. tsutsugamushi DNA extracted from infected tissues from mice and monkeys following experimental infection with Orientia showed 27-5,552 copies/µL of mouse blood, 14,448-86,012 copies/µL of mouse liver/spleen homogenate, and 3-21 copies/µL of monkey blood.

In 1993, Malawi stopped treating patients with chloroquine for Plasmodium falciparum malaria because of a high treatment failure rate (58%). In 1998, the in vitro resistance rate to chloroquine was 3% in the Salima District of Malawi; in 2000, the in vivo resistance rate was 9%. We assayed two genetic mutations implicated in chloroquine resistance (N86Y in the P. falciparum multiple drug resistance gene 1 and K76T in the P. falciparum chloroquine resistance transporter gene) in 82 P. falciparum isolates collected during studies in 1998 and 2000. The prevalence of N86Y remained similar to that in neighboring African countries that continued to use chloroquine. In contrast, the prevalence of K76T was substantially lower than in neighboring countries, decreasing significantly from 17% in 1998 to 2% in 2000 (P < 0.02). However, neither mutation was significantly associated with in vivo or in vitro resistance (P > 0.29). Withdrawal of the use of chloroquine appears to have resulted in the recovery of chloroquine efficacy and a reduction in the prevalence of K76T. However, other polymorphisms are also expected to contribute to resistance.

Leishmanaviirus (LRV) is a double-stranded RNA virus that infects the protozoa Leishmania and has been identified in numerous strains of Leishmania braziliensis and L. braziliensis guyanensis. In general, the species of Leishmania dictates disease manifestation except in the case of L. braziliensis, which is capable of causing either cutaneous or mucocutaneous leishmaniasis. We
wanted to determine 1) the quantity of LRV RNA present in a clinical sample and 2) if infection with LRV was associated with a specific disease manifestation. A real-time reverse transcriptase-polymerase chain reaction assay was used to assay clinical samples for the presence of LRV. Of 47 samples tested, 12 positive samples were obtained from patients with cutaneous lesions, lesions in the process of scarring, and cutaneous scars. This is the first study to examine the prevalence of LRV RNA within a small cohort from Brazil.

http://www.ajtmh.org/cgi/content/abstract/70/6/604

We present a polymerase chain reaction-restriction fragment length polymorphism method to simultaneously distinguish the two Anopheles gambiae M and S molecular forms and Anopheles arabiensis. This method uses different diagnostic sites than previously published methods, and it is based on the amplification of a smaller ribosomal DNA fragment. We have tested this protocol in a variety of samples from different geographic regions and various ages of preservation to ascertain the robustness of this protocol over a wide geographic window and on DNA templates of poor quality. This procedure is as efficient as previous ones in discriminating An. arabiensis from the two taxa in An. gambiae s.s. However, it performs better than others on poor quality templates such as the ones from museum collections, and poorly stored field collected material. However, it must be noted that it does not allow the simultaneous discrimination of all the species in the An. gambiae complex.

http://www.ajtmh.org/cgi/content/abstract/70/5/481

Plasmodium falciparum infection during pregnancy may cause placental malaria and subsequently low birth weight, primarily through the placental sequestration of infected red blood cells. Measuring the burden of malaria during pregnancy usually involves determining the prevalence of placental malaria infection through microscopic examination of placental blood films, a difficult and error-prone process. A number of rapid diagnostic tests (RDTs) for malaria have been developed, most of them immunochromatographic dipstick assays. However, none have been tested for the direct determination of malaria antigen in placental blood. We undertook an evaluation of the Malaria Rapid Test (MAKROmed(R)) in determining placental malaria infection. The prevalence of placental parasitemia was 22.6% by microscopy, 51.0% by a polymerase chain reaction (PCR), and 43.1% by RDT. When the PCR was used as the gold standard, RDTs had a sensitivity of 89% and a specificity of 76%. The MAKROmed RDT was highly sensitive in the detection of placental malaria, but had lower than expected specificity.

Anopheles fluviatilis, one of the major vectors of malaria in India, is a complex of at least three cryptic species provisionally designated as species S, T, and U. Identification of the cryptic species of An. fluviatilis complex is of paramount importance in disease control program due to contrasting differences in their vectorial efficiency, preference for feeding on humans, and resting behavior. Species S, T, and U are morphologically indistinguishable at any stage of their life cycle and can be identified only by the examination of species-specific fixed inversions in the polytene chromosomes. We report an allele-specific polymerase chain reaction assay for the differentiation of members of An. fluviatilis complex, which is based on differences in nucleotide sequences in D3 domain of 28S ribosomal DNA. The assay was evaluated against chromosomally examined individuals from different localities with different sympatric associations and was found to differentiate unambiguously all the members of the complex.


A cohort study involving 60 human immunodeficiency virus (HIV)-negative male transvestite commercial sex workers (CSWs) was conducted in Montevideo, Uruguay in 1999-2001. Serum samples were tested for HIV by an enzyme-linked immunosorbent assay screening with immunoblot confirmation. Six participants seroconverted for an incidence-density rate of 6.03 (95% confidence interval = 2.21-13.12) per 100 person-years. Inconsistent condom use during client sex (adjusted hazard ratio [AHR] = 6.7), during oral sex (AHR = 5.6), and at the last sexual encounter (AHR = 7.8), and use of marihuana (AHR = 5.4) were marginally associated with HIV seroconversion. Five samples were genotyped in the protease and reverse transcriptase regions; three were subtypes B and two were BF recombinants. Full genome analysis of four samples confirmed all three subtype B samples and one of the two BF recombinants. Male transvestite CSWs sustained a high rate of HIV infection. Larger prospective studies are required to better define subtypes and associated sexual and drug-related risk factors.

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We describe for distylous Turnera subulata a polygalacturonase specific to short-styled plants that is localized to the style transmitting tissue (the tissue through which pollen tubes grow). The polygalacturonase gene is linked to and may be upregulated by the S allele of the distyly locus. Because of its tissue-specific location, the polygalacturonase may be involved in the self-incompatibility response, acting in a complementary or antagonistic manner, or possibly in signalling downstream events. A pollen-specific polygalacturonase was also identified and may be a member of a small multigene family of pollen polygalacturonases. The role, if any, played by
the pollen polygalacturonase in distyly, is presently unknown.


http://www.amjbot.org/cgi/content/abstract/89/10/1697

Sequences from s6pdh, a gene that encodes sorbitol-6-phosphate dehydrogenase in the Rosaceae, are used to reconstruct the phylogeny of 22 species of Prunus. The s6pdh sequences alone and in combination with previously published sequences of the internal transcribed spacer (ITS) and the cpDNA trnL-trnF spacer are analyzed using parsimony and maximum likelihood methods. Both methods reconstructed the same phylogeny when s6pdh sequences are used alone and in combination with ITS and trnL-trnF, and the topology is in agreement with previous studies that used a larger sample size. The s6pdh sequences have about twice as many informative sites as ITS. A molecular clock is rejected for s6pdh, most likely due to greater rates of evolution in subgenera Padus and Laurocerasus than in the rest of the genus. Phylogenetic reconstruction of Prunus as determined by analysis of the combined data set suggests an early split into two clades. One is composed of subgenera Cerasus, Laurocerasus, and Padus. The second includes subgenera Amygdalus, Emplectocladus, and Prunus. Species of section Microcerasus (formerly in subgenus Cerasus) are nested within subgenus Prunus. The order of branching and relationships among early diverging lineages is weakly supported, as a result of very short branches that may indicate rapid radiation.


http://www.amjbot.org/cgi/content/abstract/89/12/2017

The amplified fragment length polymorphism (AFLP) method was used to evaluate genetic diversity and to assess genetic relationships within the section Mentha in order to clarify the taxonomy of several interspecific mint hybrids with molecular markers. To this end, genetic diversity of 62 Mentha accessions from different geographic origins, representing five species and three hybrids, was assessed. Three EcoRI/MseI AFLP primer combinations generated an average of 40 AFLP markers per primer combination, ranging in size from 50 to 500 base pairs (bp). The percentage of markers polymorphic ranged from 50% to 60% across all accessions studied. According to phenetic and cladistic analysis, the 62 mint accessions were grouped into two major clusters. Principal coordinates analysis separated species into well-defined groups, and clear relationships between species and hybrids could be described. Our AFLP analysis supports taxonomic classification established among Mentha species by conventional (morphological, cytological, and chemical) methods. It allows the assessment of phenetic relationships between species and the hybrids M. spicata and M. x piperita, largely cultivated all over the world for their menthol source, and provides new insights into the subdivision of M. spicata, based for the first time on molecular markers.


http://www.amjbot.org/cgi/content/abstract/90/10/1470
Taxonomic consensus is lacking on the Oxytropis arctica and O. campestris species complexes, two polyploid complexes found in the interior and arctic areas of Alaska. One classification has emphasized flower size, whereas flower color is considered a key diagnostic character in another classification. Our analyses of internal transcribed spacer (ITS) sequences and random amplified polymorphic DNA (RAPD) markers provided no support for either classification system. The trees generated from ITS sequences and the phenogram derived from RAPD markers suggest that most recognized taxa in the two complexes are probably polyphyletic, including O. arctica var. barnebyana, which is listed as threatened in Alaska. The only consistent pattern detected by both types of molecular markers was a geographic split dividing the northeastern arctic populations from most other populations (48.60-55.03% in AMOVA analyses). This genetic subdivision probably reflects a Pleistocene barrier formed by the northern coastal ice shield. Our molecular data, in conjunction with the previously reported variation of ploidy levels in these groups, suggest a scenario of recent and multiple origins of polyploidy. It is possible that most Alaskan populations of these two complexes are best referred to as a single taxonomic species despite morphological differentiation within the complexes.


http://www.amjbot.org/cgi/content/abstract/89/10/1709

The Hawaiian endemic mints constitute a major island radiation, displaying a remarkable diversity of floral, fruit, and vegetative features. Haplostachys and Phyllostegia have flowers associated with insect pollination, whereas Stenogyne has flowers typical of bird pollination. The three genera had been thought to be closely related to East Asian members of Lamioideae tribe Prasieae because of the fleshy nutlets borne by Phyllostegia and Stenogyne. We evaluated the origins of the Hawaiian mints using phylogenetic analyses of DNA sequence data from the plastid rbcL and trnL intron loci and the nuclear ribosomal 5S nontranscribed spacer. The Hawaiian genera were found to be monophyletic but deeply nested inside another lamiod genus, Stachys. In particular, they were found to be most closely related to a group of temperate North American Stachys from the Pacific coast, suggesting that the Hawaiian mints derived from a single colonization event from western North America to the Hawaiian Islands. Furthermore, Stachys, which contains amphiatlantic and transberingian clades, was found to be polyphyletic, with some species more closely related to Gomphostemma, Phlomidoschema, Prasium, and Sideritis than to other species of Stachys. Based on chromosomal evidence and our phylogenetic analyses, we hypothesize that the Hawaiian mints may be polyploid hybrids whose reticulate genomes predate the Hawaiian dispersal event and are derived from Stachys lineages with flowers exhibiting insect- vs. bird-pollination characteristics. Thus, the Hawaiian endemic mints may provide yet another insular system for the combined study of polyploidy, hybrid cladogenesis, and adaptive radiation.


http://www.amjbot.org/cgi/content/abstract/90/3/508

Recent advances in molecular systematics of the ferns make it possible to address long-standing questions about classification of the major fern genera, such as the worldwide genus Polystichum (Dryopteridaceae), comprising at least 200 species. In this study we examined rbcL sequences and morphological characters from 55 fern taxa: 34 were from Polystichum and 21 were from other genera in the Dryopteridaceae. We found that Phaneropherelia, possibly including Polystichopsis, is the sister group to Polystichum sensu lato (s.l.), including Cyrtomium.
Polystichum as commonly recognized is paraphyletic. Our results lead us to suggest recognizing the clade of earliest diverging Polystichum species as a distinct genus (Cyrtomidictyum) and to continue to recognize Cyrtomium as a separate genus, leaving a monophyletic Polystichum sensu stricto (s.s.). We resolved a tropical American clade and an African clade within Polystichum s.s. However, the resemblance between the once-pinnate, bulb-bearing calciphilic species found in Asia and the West Indies appears to be the result of convergent evolution. Optimizing our morphological character transformations onto the combined phylogeny suggests that the common ancestor of Polystichum s.l. and Phanerophlebia had evolved the common features of the alliance, including ciliate petiole-base scales, once-pinnate fronds, ultimate segments with scarious tips, peltate indusia, and microscales.


http://www.amjbot.org/cgi/content/abstract/91/12/2069

The New World endemic genus Zeltnera consists of 25 species mainly distributed in the western part of the United States and Mexico. Chromosome counts performed on 113 populations (24 species) reveal extensive congruence between chromosomal groups and the assemblages obtained from analyses of nuclear ribosomal DNA (ITS) and chloroplast DNA (trnL intron and trnL-F intergenic spacer) sequences. Karyological and molecular data sets support three main biogeographic groups for Zeltnera. A first and mainly unresolved cluster (n = 17 and n = 20) occurs in California, whereas two other clades are centered in the Texas region (n = 20 and n = 21) and in Mexico (n = 21 and n = 22). Under the assumption of a molecular clock, and using both dispersal and vicariance explanations for the current distribution of the respective species, the genus is thought to have a North American origin with considerable diversification in the early Pliocene (ca. 5 million years ago). Geological events, such as desert formation and mountain orogenies, have created insuperable barriers that today separate the three major and likely vicariant groups.


http://www.amjbot.org/cgi/content/abstract/90/10/1463

The tribes and subtribes of Aurantioidae, an economically important subfamily of the Rutaceae, have a controversial taxonomic history because of the lack of a phylogenetic framework. The rps16 and trnL-trnF sequences of the chloroplast were analyzed phylogenetically to construct an evolutionary history and evaluate the most recent classification system of Swingle and Reece (The Citrus Industry, volume 1 [1967]). Taxa representing tribes Citreae and Clauseneae and five of the six subtribes were sampled. Conflicts in the positions of some taxa between the rps16 and trnL-trnF trees are poorly supported. In all analyses, the Aurantioidae are monophyletic. The strict consensus tree of the combined analysis indicates that the two tribes along with the subtribes sampled are not monophyletic. The combined topology is not congruent with the widely used classification of Aurantioidae by Swingle and Reece. The tribes and subtribes are in need of revision.

The genus Arceuthobium (dwarf mistletoes, Viscaceae) comprises 42 species that parasitize hosts in Pinaceae and Cupressaceae in the Old and New Worlds. Maximum parsimony analyses were conducted on two data partitions (separately and combined): nuclear ribosomal internal transcribed spacer (ITS) sequences for all 42 currently recognized species and chloroplast trnT-L-F sequences for 34 New World species. The Old and New World species were phylogenetically distinct using ITS, thus making subgenus Arceuthobium paraphyletic. Arceuthobium pendens and A. guatemalense comprise the basalmost clade of subgenus Vaginata, characterized by the presence of flabellate secondary branching. The trnT-L-F sequences, which vary widely in length depending upon taxon, contain three times less phylogenetic signal than ITS, although homoplasy for this partition is lower. Several of the clades obtained from analysis of nuclear ITS sequences are also recovered using trnT-L-F sequences such as A. guatemalense and A. pendens, the A. rubrum group, the A. vaginatum group, and the A. campylopodum group. The ITS + trnT-L-F tree is well resolved except for four internal nodes. A revised classification of the genus is discussed that recognizes only monophyletic species that are well differentiated by molecular data.


A phylogeny of the tribe Neillieae (Rosaceae), which comprises Neillia, Stephanandra, and Physocarpus, was reconstructed based on nucleotide sequences of several regions of cpDNA, the ITS and ETS regions of rDNA, and the second intron of LEAFY, to elucidate relationships among genera and species in Neillieae and to assess the historical biogeography of the tribe. Phylogenetic analyses indicated that Physocarpus and Neillia-Stephanandra were strongly supported as monophyletic and suggested that Stephanandra may have originated by hybridization between two lineages of Neillia. Dispersal-vicariance analyses suggested that the most recent common ancestor of Neillieae may have occupied eastern Asia and western North America and that Physocarpus and Neillia-Stephanandra may have been split by an intercontinental vicariance event in the early Miocene. The biogeographic analyses also suggested that species of Neillia and Stephanandra diversified in eastern Asia, whereas in Physocarpus one dispersal event from western North America to eastern Asia occurred. Two divergent types of LEAFY sequences were found in the eastern North American species, P. opulifolius, but only one type was present in each plant. The two types of sequences may represent homeologous genes that originated by hybridization between P. capitatus and P. monogynus, both western North American species.


For nearly all species in the three genera of tribe Sinningieae (Gesneriaceae), Sinningia, Paliavana, and Vanhouttea (mostly in southeastern Brazil) plus 10 outgroups, we have sequenced six non-coding DNA regions (i.e., plastid intergenic spacers trnT-trnL, trnL-trnF, trnS-
trnG, atpB-rbcL, and introns in the trnL and rpl16 genes) and four introns in nuclear plastid-expressed glutamine synthetase gene (ncpGS). Separate and combined analyses of these data sets using maximum parsimony supported the monophyly of Sinningieae, but the genera Paliavana and Vanhouttea were found embedded within Sinningia; therefore a new infrageneric classification is here proposed. Mapping of pollination syndromes on the DNA-based trees supported multiple origins of hummingbird and bee syndromes and derivation of moth and bat syndromes from hummingbird flowers. Perennial tubers were derived from perennial stems in non-tuberous plants.


http://www.amjbot.org/cgi/content/abstract/89/7/1074

The subtribe Espeletiinae (Asteraceae, Heliantheae) comprises morphologically and ecologically diverse plants endemic to the tropical montane paramos of the Andes of Venezuela, Colombia, and Ecuador. Though the ecophysiology and ecology of this adaptive radiation have been well studied, relationships among taxa in the subtribe and between the subtribe and other taxa in the Heliantheae are poorly known. In this study, sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA are used to test previous hypotheses about the phylogenetic position of the Espeletiinae within the Heliantheae and to determine which taxa are the subtribe’s closest relatives. Gene phylogenies based on maximum parsimony analyses reveal that the Espeletiinae clade is nested well within the subtribe Melampodiinae and thus should be considered a monophyletic complex of species, not a separate subtribe. The most parsimonious gene trees suggest that the genus Ichthyothere may be the sister taxon to the Espeletia complex and that the genus Smallanthus and a species of Rumfordia are likely among the complex’s other closest living relatives. These data offer preliminary insights into the origins of this adaptive radiation and the broader phylogenetic context in which it occurred.


http://www.amjbot.org/cgi/content/abstract/89/1/145

DNA sequences from the chloroplast trnL-F region of 154 Rubiaceae and 11 outgroup taxa were analyzed cladistically. An emphasis was placed on the tribes Rondeletiaceae, Sipaneeae, and Condamineae. Sipaneeae are not close to Rondeletiaceae and belong in the Ixoroideae. There is no support for a widely distributed Rondeletiaceae in a broad sense. Instead, Rondeletiaceae sensu stricto form an almost entirely Antillean clade. Support was found for the separation of Arachnothyryx, Rogiera, Roigella, and Suberanthus from Rondeletia. The Guettardeae as well as Gonzalagunia are found close to a complex formed by Arachnothyryx, Javorkaea, and Rogiera. Condamineae, in a strict sense, belongs in the Ixoroideae. A number of Rondeletiaceae genera should be transferred to Condamineae or other parts of Ixoroideae. Support is found for an emended tribe Naucleeae, comprising several genera with spherical pseudanthia. For the first time, tribal or subfamilial affiliation based on molecular sequence data is suggested for Allenanthus, Blepharidium, Chione, Coutaportla, Dolichodelphys, Mazaea, Neobertiera, Neoblakea, Phialanthus, Phyllacanthus, Phyllomelia, Schmidtottia, and Suberanthus.

http://www.amjbot.org/cgi/content/abstract/90/5/777

DNA sequences from plastid rbcL and matK genes and the trnL-F region, as well as the nuclear ribosomal ITS region, were used to evaluate monophyly and subtribal delimitation of Cranichideae and generic relationships in Spiranthinae. Cranichideae are moderately supported as monophyletic, with Chloraeinae and Pterostylis-Megastylis indicated as their collective sisters. Within Cranichideae, Pachyplectroninae and Goodyerinae form a well-supported monophyletic group sister to a "core spiranthid" clade that includes, according to their branching order, Galeottiiellinae, Manniellinae, and a Prescottiinae-Cranichidinae-Spiranthinae subclade. Inclusion of Galeottiella in Spiranthinae, as in previous classifications, renders the latter paraphyletic to all other spiranthid subtribes. Cranichidinae and Spiranthinae (minus Galeottiella) are monophyletic and strongly supported, but Prescottiinae form a grade that includes a strongly supported prescottioid Andean clade and a weakly supported Prescottia-Cranichidinae clade sister to Spiranthinae. Well-supported major clades in Spiranthinae identified in this study do not correspond to previous alliances or the narrowly defined subtribes in which they have been divided recently. Morphological characters, especially those that have been used for taxonomic delimitation in Cranichideae, are discussed against the framework of the molecular trees, emphasizing putative synapomorphies and problems derived from lack of information or inadequate interpretation of the characters.


http://www.amjbot.org/cgi/content/abstract/91/2/274

Jamesonia and Eriosorus are two traditionally recognized fern genera in the Neotropics that together form a monophyletic group. Molecular phylogenetic analyses for this study suggest, however, that neither genus is itself monophyletic and that several independent lineages with the jamesonia morphotype have each undergone a fairly recent radiation in paramo ecosystems. A robust phylogeny was generated based on sequence data of the nuclear external transcribed spacer (ETS) of 18S-26S rDNA, the plastid gene rps4 and the intergenic spacer rps4-trnS. Several conclusions can be made concerning the evolutionary history and biogeographic patterns of the Jamesonia-Eriosorus complex: (1) "jamesonia" is polyphyletic, making "eriosorus" paraphyletic; (2) all analyses recover three major clades in the Andes; (3) two well-supported clades can be recognized, corresponding to the northern vs. central Andes; and (4) the sister taxon of the Andean radiation is the Brazilian taxon Eriosorus myriophyllus. Jamesonia is a potential example of a recent adaptive radiation because the group is characterized as being morphologically and ecologically diverse and its habitat is of recent origin.


http://www.amjbot.org/cgi/content/abstract/91/6/943

Phylogenetic relationships and biogeography of the genus Cerastium were studied using sequences of three noncoding plastid DNA regions (trnL intron, trnL-trnF spacer, and psbA-trnH
A total of 57 Cerastium taxa was analyzed using two species of the putative sister genus Stellaria as outgroups. Maximum parsimony analyses identified four clades that largely corresponded to previously recognized infrageneric groups. The results suggest an Old World origin and at least two migration events into North America from the Old World. The first event possibly took place across the Bering land bridge during the Miocene. Subsequent colonization of South America occurred after the North and South American continents joined during the Pliocene. A more recent migration event into North America probably across the northern Atlantic took place during the Quaternary, resulting in the current circumpolar distribution of the Arctic species. Molecular clock dating of major biogeographic events was internally consistent on the phylogenetic trees. The arctic high-polyploid species form a polytomy together with some boreal and temperate species of the C. tomentosum group and the C. arvense group. Lack of genetic variation among the arctic species probably indicates a recent origin. The annual life form is shown to be of polyphyletic origin.


http://www.amjbot.org/cgi/content/abstract/92/1/92

Although red pine (Pinus resinosa) generally has low or completely lacks variation for molecular markers, some variation is observed for chloroplast microsatellites (cpSSRs). We sampled and examined 10 cpSSRs for 19 populations. Analysis of these populations plus 10 previously studied populations shows that the geographic distribution of genetic diversity over the range of P. resinosa is markedly nonuniform. Although the pattern exhibits little isolation by distance, there is a region centered in northeastern New England where populations contain much greater chloroplast haplotype diversity than elsewhere. This area is band-shaped, with the longer axis nearly parallel with latitude, and very sharply delineated. The area of high diversity was buried by the Laurentide ice sheet. The geographic pattern indicates that P. resinosa is not at equilibrium, and the species has had a more complex postglacial history than typically purported for forest trees in eastern North America. The results suggest that the area of high diversity is a stable transition zone between descendants of two distinct refugia, one in the southern Appalachians and another near the North Atlantic coastline of the Wisconsinan glacial period. Plausible explanations are given that selection between two lineages, along latitudinal zones, may have maintained the transition zone.

Am. J. Clinical Nutrition (2)


http://www.ajcn.org/cgi/content/abstract/76/5/1117

Background: Compromised barrier function and intestinal inflammation are common complications of total parenteral nutrition (TPN). Objective: We tested the hypothesis that the lack of enteral nutrients in TPN might select commensal or pathogenic bacteria that use mucus as a substrate, thereby weakening the protection provided by the intestinal mucus layer. Design: Ileal
microbiota profiles of piglets fed by total enteral nutrition (TEN; n = 6) or TPN (n = 5) were compared with the use of 16S ribosomal DNA polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis and with a PCR-based method developed to specifically measure Clostridium perfringens concentrations. Ileal bacteria from TEN and TPN piglets were also examined for their ability to grow on mucin or sulfated monosaccharides. Results: Bacterial community structure was equally complex in the ileum of TEN and TPN piglets, but profiles clustered according to mode of nutrition. Sixty-two percent of total mucus-associated bacteria (100 colonies tested) in TPN compared with 33% of mucus-associated bacteria (100 colonies tested) in TEN ileal samples grew on mucin. Bacteria capable of using sulfated monosaccharides were also enriched in TPN samples. C. perfringens, an opportunistic pathogen, was specifically enriched in the TPN ileum (P < 0.05). These results were corroborated by cultivation-based studies that showed rapid growth of C. perfringens on mucin-based substrates. Conclusions: Mucolytic potential is widespread among intestinal bacteria. Mucolytic bacteria in general and C. perfringens in particular were selected when enteral nutrients were withheld in this TPN piglet model. Similar enrichment processes may occur in humans nourished by TPN and may thereby contribute to intestinal dysfunction.


http://www.ajcn.org/cgi/content/abstract/77/4/809

Background: The association between polymorphisms in the scavenger receptor class B type I (SRB-I) gene and variations in basal plasma concentrations of cholesterol in humans has recently been described. Objective: The objective of the study was to determine whether the exon 1 variant (G[-&gt;]A) at the SRB-I gene is associated with the lipid response to the content and quality of dietary fat in healthy subjects. Design: We studied 97 healthy volunteers with exon 1 polymorphism [65 homozygous for allele 1 (1/1) and 32 heterozygous for allele 2 (1/2)]. Both groups consumed 3 diets lasting 4 wk each. The first was a saturated fatty acid (SFA)-rich diet (38% fat, 20% SFA), which was followed by a carbohydrate (Cho)-rich diet (30% fat, < 10% SFA, 55% carbohydrate) or a monounsaturated fatty acid (MUFA), olive oil-rich diet (38% fat, 22% MUFA) according to a randomized crossover design. At the end of each dietary period, plasma concentrations of triacylglycerol and of total, LDL, and HDL cholesterol were measured. Results: Carriers of the 1/2 genotype had a trend toward higher concentrations of LDL cholesterol (P < 0.11) after the SFA-rich diet than did those who were homozygous for 1/1. Carriers of the mutation showed a significantly greater (P = 0.007) decrease in LDL-cholesterol concentrations (-23%) in changing from an SFA-rich diet to a Cho-rich diet than did noncarriers of the mutation (-16%). Conclusion: Carriers of the minority allele, 1/2, are more susceptible to the presence of SFA in the diet because of a greater increase in LDL cholesterol.

Am. J. Epidemiol. (1)

The association between androgen receptor gene polymorphisms and benign prostatic hyperplasia was investigated among 510 men randomly selected from Olmsted County, Minnesota. From 1990 through 2000, lower urinary tract symptom severity was assessed by the American Urological Association Symptom Index (AUASI), and peak urinary flow rate, prostate volume, and serum prostate-specific antigen level were measured. Androgen receptor CAG and GGN genotyping was performed. A CAG repeat length of <21 was associated with an enlarged prostate (hazard ratio (HR) = 1.4, 95% confidence interval (CI): 1.0, 1.9) and a serum prostate-specific antigen level >1.4 ng/ml (HR = 1.5, 95% CI: 1.1, 2.0). A GGN repeat length of <16 was associated with an AUASI >7 (HR = 1.6, 95% CI: 1.1, 2.3) and a serum prostate-specific antigen level >1.4 ng/ml (HR = 1.5, 95% CI: 1.0, 2.3). Having <21 CAG repeats and <16 GGN repeats compared with having neither was associated with an enlarged prostate (HR = 2.5, 95% CI: 1.5, 4.2), a serum prostate-specific antigen level >1.4 ng/ml (HR = 2.8, 95% CI: 1.6, 4.7), a peak flow rate <12 ml/second (HR = 1.9, 95% CI: 1.1, 3.4), and an AUASI >7 (HR = 1.6, 95% CI: 1.0, 2.7). Androgen receptor gene polymorphisms may have a potential role in the pathogenesis of benign prostatic hyperplasia.

Am. J. Geriatr. Psychiatry (1)


OBJECTIVE: A systematic genome survey was initiated to identify loci that affect the likelihood of reaching age 90 with preserved cognition. This communication describes the clinical characterization and comparison of the experimental groups, validation of the experimental method, and results for the Y chromosome. METHODS: The genome survey was conducted at 10 cM resolution for simple sequence tandem repeat polymorphisms (SSTRPs) that identify genes for successful aging by virtue of linkage disequilibrium. Efficiency was enhanced by genotyping pools of DNA from 100 cognitively intact elders (50 men/50 women) and 100 young (age 18-25 years) adults matched for sex, race, ethnicity, and geographic location. RESULTS: Elders (94 nonagenarians, 6 centenarians) manifested preserved cognition, as reflected by clinical and psychometric assessments; "good" average capacity to carry out their activities of daily living; and the majority were living independently despite multiple medical conditions. None had a history of mental disorders in early or middle adulthood, only one was a current smoker, and 80% consumed alcohol less than once each month. The genome survey method detected the expected elevation of the APOE (epsilon)2 allele frequency, and reciprocal reduction in the (epsilon)4 frequency, among the elders, compared with the young adults. It also detected significant differences in the allelic distributions of DYS389 and DYS390, which are separated by only 2.6 Mb near the centromere of Yq. CONCLUSIONS: These results suggest that several behavioral and genetic factors may contribute to the likelihood of achieving exceptional longevity with preserved cognition.
Eotaxin is a critical chemokine eliciting migration of eosinophils and basophils in the pathogenesis of bronchial asthma. Recent studies have shown that the specific receptor for eotaxin, CCR3, is expressed in bronchial epithelial cells. Although mitogen-activated protein (MAP) kinases are involved in diverse cell functions of bronchial epithelial cells, their role in eotaxin signaling is unknown. In this study, we studied the activation and functional relevance of MAP kinases in bronchial epithelial cells stimulated with eotaxin. Eotaxin (1-100 nM) induced tyrosine/threonine phosphorylation and activation of extracellular regulated kinase (ERK) 1/2 and p38 in NCI-H292 cells and normal human bronchial epithelial cells. The phosphorylation of these MAP kinases was detectable after 30 s, and peaked at 5 min. Eotaxin stimulated production of interleukin-8 and granulocyte macrophage colony-stimulating factor. Pretreatment of Compound X (a specific CCR3 antagonist), pertussis toxin, genistein, and wortmannin reduced the MAP kinase phosphorylation and cytokine production. The eotaxin-induced cytokine production was inhibited by specific inhibitors for MAP/ERK kinase (PD98059) and p38 MAP kinase (SB202190). These results suggest that both ERK1/2 and p38 MAP kinase activated by eotaxin have a critical role in the pathogenesis of asthma.


Many military personnel are at risk of lung damage or systemic toxicity as a result of exposure to the jet fuel JP-8. We have now used microarray analysis to characterize changes in the gene expression profile of lung tissue induced by exposure of rats to JP-8 at a concentration of 171 or 352 mg/m3 for 1 h/d for 7 d, with the higher dose estimated to mimic the level of occupational exposure in humans. The expression of 56 genes was significantly affected by a factor of \( \leq 0.6 \) or \( \geq 1.5 \) by JP-8 at the low dose. Eighty-six percent of these genes were downregulated by JP-8. The expression of 66 genes was similarly affected by JP-8 at the higher dose, with the expression of 42% of these genes being upregulated. Prominent among the latter genes was that for the centrosome-associated protein \( \{ \text{gamma}\} \)-synuclein, whose expression was consistently increased. The expression of various genes related to antioxidant responses and detoxification, including those for glutathione S-transferases and cytochrome P450 proteins, were also upregulated. The microarray data were confirmed by quantitative RT-PCR analysis. Our extensive data set may thus provide important insight into the pulmonary response to occupational exposure to JP-8 in humans.

Asthma is a chronic inflammatory disease of the airways. Mast cell-derived cytokines may mediate both airway inflammation and remodeling. It has also been shown that fibroblasts can be the source of proinflammatory cytokines. In the human airways, mast cell-fibroblast interactions may have pivotal effects on modulating inflammation. To study this further, we cocultured normal human lung fibroblasts (NHLF) with a human mast cell line (HMC-1) and assayed for production of interleukin (IL)-6, an important proinflammatory cytokine. When cultured together, NHLF/HMC-1 contact induced IL-6 secretion. Separation of HMC-1 and NHLF cells by a porous membrane inhibited this induction. HMC-1-derived cellular membranes caused an increase in IL-6 production in NHLF. Activation of p38 MAPK was also seen in cocultures by Western blot, whereas IL-6 production in cocultures was significantly inhibited by the p38 inhibitor SB203580. IL-6 production in cocultures was minimally inhibited by a chemical inhibitor of nuclear factor-(kappa)B (Bay11), indicating that nuclear factor-(kappa)B may have a minimal role in signaling IL-6 production in mast cell/fibroblasts cocultures. Blockade of inter-cellular adhesion molecule-1, tumor necrosis factor-RI, and surface IL-1{beta} with neutralizing antibodies failed to significantly decrease IL-6 production in our coculture, indicating that other receptor-ligand associations may be responsible for this activation. These novel studies reveal the importance of cell-cell interactions in the complex milieu of airway inflammation.


http://ajrcmb.atsjournals.org/cgi/content/abstract/32/3/201

Respiratory tract infections result in wheezing in a subset of patients. Mycoplasma pneumoniae is a common etiologic agent of acute respiratory infection in children and adults that has been associated with wheezing in 20-40% of individuals. The current study was undertaken to elucidate the host-dependent pulmonary and immunologic response to M. pneumoniae respiratory infection by studying mice with different immunogenetic backgrounds (BALB/c mice versus C57BL/6 mice). After M. pneumoniae infection, only BALB/c mice developed significant airway obstruction (AO) compared with controls. M. pneumoniae-infected BALB/c mice manifested significantly elevated airway hyperresponsiveness (AHR) compared with C57BL/6 mice 4 and 7 d after inoculation as well as BALB/c control mice. Compared with C57BL/6 mice, BALB/c mice developed worse pulmonary inflammation, including greater peribronchial infiltrates. Infected BALB/c mice had significantly higher concentrations of tumor necrosis factor-(alpha), interferon-(gamma), interleukin (IL)-1(bet a), IL-6, IL-12, KC (functional IL-8), and macrophage inflammatory protein 1(alpha) in the bronchoalveolar lavage fluid compared with infected C57BL/6 mice. No differences in IL-2, IL-4, IL-5, IL-10, and granulocyte/macrophage colony-stimulating factor concentrations were found. The mice in this study exhibited host-dependent infection-related AO and AHR associated with chemokine and T-helper type (Th)1 pulmonary host response and not Th2 response after M. pneumoniae infection.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/5/720

We investigated the mechanisms of endogenous nitric oxide (NO) modulation of lung sodium (Na+) transport. C57BL/6 mice injected intraperitoneally with the specific inducible NO synthase (iNOS) inhibitor 1400W (10 mg/kg every 8 h for 72 h) exhibited decreased alveolar nitrate levels and Na+-dependent amiloride-sensitive alveolar fluid clearance as compared with mice injected with vehicle. Similarly, pretreatment of mouse tracheal epithelial cells with 1400W abolished the
inhibitory effects of amiloride on their Na+ short circuit currents. On the other hand, mouse tracheal epithelial cells pretreated with 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, a specific inhibitor of guanylate cyclase, had lower levels of cGMP, but normal values of amiloride-sensitive Na+ currents. Amiloride also inhibited whole-cell Na+ currents across A549 cells treated with vehicle (Ki = 249 nM), but had no effect in A549 cells treated with 1400W. Western blotting studies showed significantly lower levels of (alpha) and (gamma)ENaC in lung tissues and alveolar type II (ATII) cells from iNOS-/- as well as iNOS+/+ mice treated with 1400W, as compared with the corresponding values from vehicle-treated iNOS+/+ mice. Similar values for ratios of (alpha), (beta), and (gamma)ENaC to gapdh were obtained by real-time polymerase chain reaction for iNOS+/+ mice and iNOS-/- mice. We concluded that NO derived from iNOS under basal conditions is necessary for amiloride-sensitive Na+ transport across lung epithelial cells and modulates the amount of (alpha) and (gamma)ENaC via post-transcriptional, cGMP-independent mechanisms.


http://ajrcmb.atsjournals.org/cgi/content/abstract/28/5/563

Tissue structural cells are known in some situations to play a role in the presentation of antigen and in immunoregulation. We assessed the expression of B7 homologs, known to be involved in antigen presentation and lymphocyte costimulation, in human airway epithelial cells. Flow cytometry performed on the airway epithelial cell line BEAS-2B, as well as primary bronchial epithelial cells (PBEC), showed that B7-H2 was constitutively expressed on both BEAS-2B and PBEC, whereas B7-1 and B7-2 were undetectable on either epithelial cell type. B7-H2 expression was confirmed by Western blot using a specific antibody. Stimulation with various cytokines, including tumor necrosis factor-(alpha), interferon-(gamma), and interleukin-4, slightly downregulated B7-H2 expression detected by flow cytometry, but did not significantly alter the apparent level of protein as assessed by Western blotting. Northern blotting detected mRNA for B7-H2 and B7-1, but not B7-2. B7-H2 was cloned from BEAS-2B cells and the sequence verified. Expression of B7-H2 mRNA was detected by real-time reverse transcriptase-polymerase chain reaction in PBEC from three independent donors. Immunohistochemical analysis of airway derived from autopsies revealed expression of B7-H2 in human airway epithelial cells. These results demonstrate that airway epithelial cells express the costimulatory molecule B7-H2, and suggest the possibility that B7-H2 may participate in antigen presentation by epithelial cells.


http://ajrcmb.atsjournals.org/cgi/content/abstract/31/3/283

Mast cells play pivotal roles in immunoglobulin (Ig) E-mediated airway inflammation, expressing interleukin (IL)-13 and monocyte chemoattractant protein-1 (MCP-1), which in turn regulate IgE synthesis and/or inflammatory cell recruitment. The molecular effects of IL-1(beta) on cytokine expression by human mast cells (HMC) have not been studied well. In this report, we provide evidence that human umbilical cord blood-derived mast cells (CBDMC) and HMC-1 cells express the type 1 receptor for IL-1. We also demonstrate that IL-1(beta) and tumor necrosis factor-(alpha) are able to induce, individually or additively, dose-dependent expression of IL-13 and MCP-1 in these cells. The induction of IL-13 and MCP-1 gene expression by IL-1(beta) was accompanied by the activation of IL-1 receptor-associated kinase and translocation of the
transcription factor, nuclear factor (NF) \((\kappaappa)B\) into the nucleus. Accordingly, Bay-11 7082, an inhibitor of NF-\((\kappaappa)B\) activation, inhibited IL-1\{(beta\)-induced IL-13 and MCP-1 expression. IL-1\{(beta\) also induced IL-13 promoter activity while enhancing the stability of IL-13 messenger RNA transcripts. Dexamethasone, a glucocorticoid, inhibited IL-1\{(beta\)-induced nuclear translocation of NF-\((\kappaappa)B\) and also the secretion of IL-13 from mast cells. Our data suggest that IL-1\{(beta\) can serve as a pivotal costimulus of inflammatory cytokine synthesis in human mast cells, and this may be partly mediated by IL-1 receptor-binding and subsequent signaling via nuclear translocation of NF-\((\kappaappa)B\). Because IL-1\{(beta\) is a ubiquitously expressed cytokine, these findings have important implications for non-IgE-mediated signaling in airway mast cells as well as for innate immunity and airway inflammatory responses, such as observed in extrinsic and intrinsic asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/1/51

Mechanical ventilation has been shown to cause ventilator-induced lung injury (VILI), probably by overdistending or stretching the lung. Hyaluronan (HA), a component of the extracellular matrix, in low molecular weight (LMW) forms has been shown to induce cytokine production. LMW HA is produced by hyaluronan synthase 3 (HAS 3). We found that HAS 3 mRNA expression was upregulated and that LMW HA accumulated in an animal model of VILI. We hypothesized that stretch-induced LMW HA production that causes cytokine release in VILI was dependent on HAS 3 mRNA expression. We explored this hypothesis with in vitro lung cell stretch. Cell stretch induced HAS 3 mRNA expression and LMW HA in fibroblasts. Nonspecific inhibitors of HAS 3 (cyclohexamide and dexamethasone), a nonspecific inhibitor of protein tyrosine kinases (genistein), and a janus kinase 2 inhibitor (AG490) blocked stretch-induced HAS 3 expression and synthesis of LMW HA. Stretch-induced LMW HA from fibroblasts caused a significant dose-dependent increase in interleukin-8 production both in static and stretched epithelial cells. These results indicated that de novo synthesis of LMW HA was induced in lung fibroblasts by stretch via tyrosine kinase signaling pathways, and may play a role in augmenting induction of proinflammatory cytokines in VILI.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/1/105

To examine the effects of acid exposure with moderate acidity (pH 3.0-5.0) on bactericidal activity of airway surface liquid (ASL), ASL was collected by washing the surface of primary cultures of human tracheal epithelial cells 24 h after treatment with phosphate-buffered saline (PBS) adjusted to a pH of 3.0, 4.0, or 5.0. In all ASL, bactericidal activity was sensitive to sodium concentration. Escherichia coli (500 colony forming units [CFU]) was incubated in ASL, and the number of surviving bacteria was examined. The number of surviving bacteria in ASL from cultured cells with acid exposure at pH 3.0-5.0 was significantly higher than that in control ASL. The minimum inhibitory dilution ratio of ASL against 500 CFU of E. coli was also examined by microdilution assays. According to this assay, the bactericidal activity in ASL with acid challenge at a pH of 3.0 was less than half of that in control ASL. Reverse transcription-polymerase chain reaction and Western blot analysis showed that the production of mRNA and protein of human [beta]-defensin (HBD)-1 were significantly decreased by acid exposure at pH 3.0-5.0. In contrast, acid exposure did not change the production of mRNA and protein of HBD-2 and [beta]-actin mRNA. These results indicate that acid exposure, even with moderate acidity, may inhibit the
production of bactericidal molecules, including HBD-1, in airway epithelial cells. Acid exposure may reduce bactericidal activity of ASL in human airway epithelial cells and may increase susceptibility of the airway to bacterial infection.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/3/348

Pulmonary inflammation increases nitric oxide (NO) production via inducible nitric oxide synthase (iNOS). This study was performed to determine some of the factors that affect the availability of the NOS substrate, L-arginine (L-arg), in the intact lung subjected to silica-induced inflammation. Nitrate production, as an index of NO production, was significantly greater in silica-exposed lungs (53.5 +/- 12.1 nmol/90 min) compared with controls (22.5 +/- 5.1 nmol/90 min, P < 0.05). This was accompanied by greater (P < 0.001) 90-min [3H]L-arg uptake (62 +/- 3% control, 82 +/- 1% silica), a significantly (P < 0.005) increased permeability-surface area product for L-arg (0.28 +/- 0.05 ml/min control, 0.63 +/- 0.07 ml/min silica), and a significantly (P < 0.001) increased urea production (1.16 +/- 0.08 [micro]mol/90 min control, 1.77 +/- 0.06 [micro]mol/90 min silica). There was no difference in eNOS protein between groups and eNOS mRNA was not detectable in either group, whereas silica exposure resulted in the appearance of both iNOS protein and mRNA. Silica exposure increased CAT-1 and CAT-2 mRNA ~ 8-fold compared with controls. We conclude that the increase in NO production in silica-exposed lungs was associated with increased L-arg uptake from the vasculature, presumably resulting from increased CAT-1 and CAT-2, and by increased L-arg metabolism via arginase.

Keratinocyte growth factor (KGF or FGF-7) stimulates alveolar type II cell proliferation, but little is known about the signaling pathways involved. We investigated the role of the ERK (p42/44 mitogen activated protein [MAP] kinase) and phosphatidylinositol 3-OH kinase (PI3 kinase) pathways on alveolar type II cell proliferation and differentiation. Rat type II cells were cultured on tissue culture plastic and Matrigel in the presence or absence of KGF and specific chemical inhibitors PD98059, LY294002, and rapamycin at various concentrations. Proliferation was measured by thymidine incorporation and DNA quantitation, and differentiation was measured by expression of surfactant protein A and alkaline phosphatase. We demonstrate that KGF activates distal effectors of the PI3 kinase pathway, PKB/Akt, and p70S6 kinase, as well as p42/44 MAP kinase proteins. Inhibition of these pathways with PD98059, LY294002, or rapamycin inhibited type II cell proliferation but had no significant effect on differentiation. KGF did not activate the c-Jun kinase or p38 MAP kinase pathways. We conclude that the p42/44 MAP kinase and PI3 kinase pathways are important in regulating alveolar type II cell proliferation in response to KGF.


Extracellular matrix (ECM) expansion contributes to airway remodeling in asthma. This study examines the effect of leukotriene D4 (LTD4), combined with epidermal growth factor (EGF), on proteoglycan synthesis by cultured human bronchial smooth muscle cells (BSMCs). LTD4 plus EGF stimulated proliferation of BSMCs with increased versican synthesis. Further, versican mRNA splice variants, V0 and V1, were differently regulated in BSMCs by LTD4 plus EGF. Synthesis of [35S]-methionine labeled versican V0, as a percentage of total versican, was doubled. This upregulation was confirmed by Western analysis. Synthetic changes were paralleled by alterations in versican V0 mRNA. The effects of LTD4 and EGF on proteoglycan synthesis were inhibited by montelukast. Similar upregulation of versican V0 was observed in arterial smooth muscle cells (ASMCs) stimulated with LTD4 plus EGF as measured by western and reverse transcriptase-polymerase chain reaction analyses. Changes in ECM in the asthmatic airway may parallel those in atherosclerotic lesions where proliferating ASMCs synthesize a versican-rich expanded ECM. Inhibition of these processes could lead to reduced tissue expansion in the early phases of asthma progression.


The ADAM (A Disintegrin and Metalloprotease) family of Zn++-dependent metalloproteases are multidomain proteins involved in diverse cellular activities. Polymorphic variation in ADAM33 is strongly associated with asthma and bronchial hyperresponsiveness. Identification of those isoforms of ADAM33 that are expressed in airways is fundamental to dissecting the role of ADAM33 in asthma. Analysis of primary human airways fibroblasts has shown the presence of a number of alternatively spliced forms of ADAM33, including one encoding a putative secreted variant, and many transcripts lacking the metalloproteinase domain. The relative abundance of these transcripts has been quantified using reverse transcription real-time polymerase chain reaction, in both nuclear and cytoplasmic fractions of RNA. These results demonstrate that a
number of splice variants of ADAM33 are transported into the cytoplasm. Ninety percent of
ADAM33 mRNA is retained in the nucleus and the subtle differences in the composition of
nuclear and cytoplasmic RNA suggest important events in the splicing and selection of ADAM33
transcripts. Western blot analysis confirmed that several protein isoforms of ADAM33 are
expressed in primary airways fibroblasts. These findings demonstrate that ADAM33 exists in
multiple isoforms, suggesting that it is a complex molecule that plays multiple roles within
mesenchymal cells.

Ricci, A., L. Felici, et al. (2004). "Neurotrophin and Neurotrophin Receptor Protein Expression in the

http://ajrcmb.atsjournals.org/cgi/content/abstract/30/1/12

Neurotrophins (NTs) promote survival and differentiation of central and peripheral neurons, and
display several activities also in non-neuronal cells. Human lungs synthesize and release NTs,
which are probably involved in the pathophysiology of pulmonary disturbances. In this article the
expression and anatomic localization of nerve growth factor, brain-derived neurotrophic factor,
and NT-3 and of corresponding high-affinity receptors TrkA, TrkB (full-length and truncated [TR-]
isoforms), TrkC, and of the low-affinity p75 receptor, were assessed in surgical samples from
adult human lung by reverse transcriptase-polymerase chain reaction, Western blot, and
immunohistochemistry. NTs and their cognate receptor mRNA and protein transcripts were
detected by reverse transcriptase-polymerase chain reaction and immunoblotting, respectively,
nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) mRNA and
corresponding protein transcripts being the most expressed. High levels of TrkB-[TR-] mRNA and
of its protein transcript were also demonstrated, whereas a low expression of p75 mRNA and of
corresponding protein transcript were found. Microanatomic analysis of immunohistochemical
study revealed that bronchial epithelial cells were immunoreactive for different NTs, with a higher
intensity of BDNF immune staining compared with other NTs, but did not express NT receptor
immunoreactivity. Alveolar cells were immunoreactive for TrkA and TrkC receptor protein, but did
not display immunoreactivity for NTs or other receptors investigated. Gland cells expressed NT
and high-affinity NT receptor immunoreactivity, but not p75 receptor immunoreactivity. NT and
low-affinity receptor immunoreactivity was observed within neurons and satellite cells of
parasympathetic ganglia as well as in nerve fiber-like structures supplying the bronchopulmonary
tree. An obvious immunoreactivity for NTs and NT receptor protein was also observed in
intrapulmonary branches of pulmonary artery. Pulmonary lymphocytes and macrophages express
nerve growth factor and high-affinity NT receptor immunoreactivity. The role of NTs in non-
neuronal tissue including lung has not been clarified yet. The widespread expression of NTs and
their receptors in different components of the lung suggests that these factors may contribute to
regulate cell function in human lung.

Neutrophils and Prevents Lung Injury in Endotoxin-Induced Airway Inflammation." Am. J. Respir.

http://ajrcmb.atsjournals.org/cgi/content/abstract/28/2/199

We investigated the pharmacologic effects of the antioxidant Vitamin E (\{\alpha\}-tocopherol
[\{\alpha\}-toc]) in airway inflammation induced by inhaled endotoxin. A preparation of \{\alpha\}-toc
incorporated in liposomes was administered intraperitoneally in mice 1 h after exposure of
aerosolized endotoxin. Injection of 50 mg \{\alpha\}-toc/kg significantly decreased the number of
neutrophils in airspaces and prevented lung injury, monitored both as decreased lactate
dehydrogenase activity in airways and reduced lung edema when compared with animals treated
with plain liposomes. Immunofluorescence staining of lung tissue revealed that treatment with \(\alpha\)-toc decreased the number of neutrophils in lung interstitium, whereas the number in lung blood vessels and peripheral blood did not differ between mice treated with \(\alpha\)-toc and control mice. Our results indicate that \(\alpha\)-toc downmodulates the migration of neutrophils across the endothelial barrier, but in contrast to strong anti-inflammatory drugs such as corticosteroids, without inhibition of transcription factors involved in the early inflammatory response (nuclear factor-(kappa)B/activator protein-1). Neither was the endotoxin-induced expression of proinflammatory cytokines in lung tissue downregulated. Treatment with a combination of \(\alpha\)-toc and a suboptimal dose of 0.5 mg/kg dexamethasone enhanced the effect, suggesting that \(\alpha\)-toc, in combination with low doses of corticosteroids, might be effective for therapeutic treatment of acute lung injury.


http://ajrcmb.atsjournals.org/cgi/content/abstract/31/1/122

Beryllium (Be) presentation to CD4+ T cells from patients with chronic beryllium disease (CBD) results in T cell activation, and these Be-specific CD4+ T cells undergo clonal proliferation and T-helper 1-type cytokine production. In exposed workers, genetic susceptibility to this granulomatous disorder is associated with particular HLA-DPB1 alleles. We hypothesized that these HLA-DP molecules could mediate Be-stimulated tumor necrosis factor-(\(\alpha\)) (TNF-(\(\alpha\))) messenger RNA (mRNA) and protein production. Using intracellular cytokine staining, we found that treatment with an anti-HLA-DP, but not anti-HLA-DR, monoclonal antibody inhibited Be-stimulated TNF-(\(\alpha\)) expression in lung CD3+ CD4+ T cells. This monoclonal antibody also blocked Be-specific T cell proliferation, increased production of TNF-(\(\alpha\)) mature-mRNA transcripts, and increased TNF-(\(\alpha\)) protein production by Be-stimulated CBD peripheral blood mononuclear cells and bronchoalveolar lavage (BAL) cells. The Be-stimulated upregulation of TNF-(\(\alpha\)) mature-mRNA levels with TNF-(\(\alpha\)) protein production was a unique property of CBD BAL cells, and did not occur in BAL cells from Be-sensitized patients without CBD, or sarcoidosis BAL cells. This study identifies HLA-DP as a regulatory component in the activation of T cell receptors on Be-specific CD4+ T cells from CBD patients resulting in proliferation and proinflammatory cytokine production.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/4/519

Human prostasin is a membrane-anchored serine peptidase hypothesized to regulate lung epithelial sodium transport. It belongs to a unique family of genes on chromosome 16p11.2/13.3. Here we describe genomic cloning, promoter analysis, and expression of prostasin's mouse ortholog. The 4.3-kb mouse prostasin gene (prss8) has a six-exon organization identical to human prostasin. Prss8 spans two signal tagged-sites localized to chromosome 7. Multiple mRNA transcripts arise from two consensus initiator elements of a TATA-less promoter and an alternatively spliced, 5' untranslated region intron. Reporter assay establishes that the initiator elements and a GC-rich domain comprise the core promoter and identifies 5' flanking regions with strong enhancer and repressor activity. The 3' untranslated region overlaps the 3' untranslated region of the Myst1 gene oriented tail-to-tail at this locus. Prss8 is highly transcribed in pancreas, kidney, submaxillary gland, lung, thyroid, prostate, and epididymis, and is developmentally regulated. Using selective riboprobes and antibodies to mouse prostasin, we localized its expression to lung airway epithelial and alveolar type II cells and kidney cortical tubule epithelium.
Mouse prostasin highly resembles its human ortholog in gene organization and tissue specificity, including strong expression in pulmonary epithelium, suggesting that mice will be useful for probing prostasin's functions in vivo.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/4/484

Cytokines derived from lymphocytes are believed to play key roles in a variety of diseases, including airway diseases such as asthma. The current study was designed to evaluate the hypothesis that cytokines derived from Th2 cells, interleukin (IL)-4 and IL-13, might contribute to tissue remodeling by modulating the production of transforming growth factor (TGF)-[beta]. In addition, the ability of interferon (IFN)-[gamma], a cytokine derived from Th1 cells that can antagonize many effects of IL-4 and IL-13, was also assessed for its effects on TGF-[beta] production. IL-4 and IL-13 both stimulated production of TGF-[beta]2 release from human bronchial epithelial cells in a time- and concentration-dependent manner. Both with and without acidification, TGF-[beta]2 were detected. Neither TGF-[beta]1 nor TGF-[beta]3 was released. In contrast to the stimulatory effect on human bronchial epithelial cells, neither IL-4 nor IL-13 stimulated release of any TGF-[beta] isoform from human lung fibroblasts. IFN-[gamma] reduced both basal, IL-4-, and IL-13-stimulated release of TGF-[beta]2 in human bronchial epithelial cells. The stimulatory effects of IL-4 and IL-13 and the inhibitory effect of IFN-[gamma] on TGF-[beta]2 release were paralleled by mRNA levels, as assessed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). In summary, the Th2-derived cytokines, IL-4 and IL-13, can stimulate production of TGF-[beta] from airway epithelial cells but not from lung fibroblasts. IFN-[gamma], in contrast, can inhibit TGF-[beta]2 release both under basal conditions and following IL-4 or IL-13 stimulation. The ability of these cytokines to modulate TGF-[beta] release may contribute to both normal airway repair and to the development of subepithelial fibrosis in asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/31/1/92

Hyaluronan (HA) is an important constituent of the extracellular matrix and accumulates during inflammatory lung diseases like asthma. Little is known about the factors that regulate HA synthesis by lung cells. Accordingly, we investigated the effect of T-helper 1 (TH1) and 2 (TH2) cytokines and the anti-inflammatory agents fluticasone and salmeterol on HA synthesis in human lung fibroblasts. Interleukin-1[beta] (IL-1[beta]) and tumor necrosis factor (TNF)-[alpha] were the most potent stimulators of HA synthesis and when combined, caused synergistic increases in HA accumulation. Time-course analysis of HA accumulation and [3H]-glucosamine incorporation into HA demonstrated continued synthesis over the 24 h of stimulation. Peak synthesis at 6-12 h coincided with an increased proportion of high molecular weight HA. Reverse transcriptase polymerase chain reaction (RT-PCR) revealed that IL-1[beta] and TNF-[alpha] induced HA synthase-2 messenger RNA (mRNA) 3 h following stimulation and remained elevated throughout the 24-h stimulation period. Fluticasone inhibited IL-1[beta] and TNF-[alpha] induced HA synthesis (44.5%) whereas salmeterol had no effect. When combined, fluticasone and salmeterol inhibited HA synthesis to a greater extent (85.2%). Further, fluticasone attenuated IL-1[beta] and TNF-[alpha] stimulated hyaluronan synthase-2 messenger RNA (mRNA), and the addition of salmeterol cooperatively enhanced this inhibition. These results indicate that enhanced synthesis of HA by the proinflammatory cytokines IL-1[beta] and TNF-[alpha] can be abrogated by specific
corticosteroid and β2 blocker combinations shown to be effective in the treatment of asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/2005-0034OCv1

IL-25, a novel Th2 cytokine, is capable of amplifying allergic inflammation. We investigated the modulation of nuclear factor-(kappa)B (NF-(kappa)B) and mitogen-activated protein kinases (MAPK) pathways in IL-25-activated eosinophils, the principal effector cells of allergic inflammation, for the in vitro release of chemokines including monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-8, and macrophage inflammatory protein (MIP)-1α, and inflammatory cytokine IL-6. Gene expression of chemokines and IL-6 was evaluated by RT-PCR and concentrations of chemokines and cytokine were measured by cytokine protein array, cytometric bead array and ELISA. NF-(kappa)B, c-Jun amino-terminal kinase (JNK) and p38 MAPK activities in eosinophils were assessed by electrophoretic mobility shift assay and Western blot. IL-25 was found to up-regulate the gene expression of chemokines MCP-1, MIP-1α, IL-8 and cytokine IL-6 in eosinophils; and significantly increase the release of the above chemokines and IL-6 from eosinophils. IL-25 could also activate the JNK, p38 MAPK and NF-(kappa)B activities of eosinophils, while inhibitor of IκB-α phosphorylation (BAY11-7082), JNK (SP600125) and p38 MAPK (SB203580) could suppress the release of IL-8, MIP-1α, MCP-1 and IL-6. Together, the above results showed that the induction of MCP-1, MIP-1α, IL-8 and IL-6 in IL-25 activated eosinophils are regulated by JNK, p38 MAPK and NF-(kappa)B pathways.


http://ajrcmb.atsjournals.org/cgi/content/abstract/29/1/133

We investigated the intracellular signaling mechanisms for cytokine interleukin (IL)-3, IL-5, or granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced expression of adhesion molecules including very late antigen 4 (CD49 d), macrophage antigen-1 (CD11b), leukocyte function-associated antigen-1 (CD11a/CD18), intercellular adhesion molecule (ICAM)-1, and ICAM-3 on eosinophils. The expression of adhesion molecules and nuclear factor (NF)-(kappa)B pathway was measured by flow cytometry and cDNA expression array, respectively. The phosphorylation of inhibitor (kappa)B-α and p38 mitogen-activated protein kinase (MAPK) was detected by Western blot, whereas NF-(kappa)B activity was measured by electrophoretic mobility shift assay. IL-3, IL-5, and GM-CSF could enhance p38 MAPK and NF-(kappa)B activity and induce ICAM-1, CD11b, and CD18 expressions on eosinophils. They could suppress ICAM-3 expression, but had no effect on CD49 d expression. Either SB 203580 or MG-132 was able to offset the cytokine-induced expression of ICAM-1. Only SB 203580 could reverse the effect on CD11b, CD18, and ICAM-3 expressions. Therefore, the expression of ICAM-1 might involve both p38 MAPK and NF-(kappa)B activities, whereas the regulation of CD11b, CD18, and ICAM-3 expressions might be mediated through p38 MAPK but not NF-(kappa)B. These cytokines therefore play a crucial role, via the p38 MAPK and NF-(kappa)B pathways, in the expression of important adhesion molecules on eosinophils in allergic inflammation.

http://ajrcmb.atsjournals.org/cgi/content/abstract/30/4/428

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease caused by mutations that affect the proper function of cilia. Recently, deletion of DNA polymerase (lambda) (Poll) in mice produced a phenotype characteristic of PCD (Kobayashi et al., 2002, Mol. Cell. Biol. 22:2769-2776). Because it is unclear how a mutation in a DNA polymerase would result in a specific defect in axonemes, the targeting construct was examined further. Analysis of the genomic region surrounding the Poll gene revealed an uncharacterized gene, named Dpcd, that is predicted to be transcribed from the opposite strand relative to Poll. The deletion of Poll would also remove the first exon of Dpcd. Because it is possible that the PCD phenotype observed is due to the absence of either gene, the expression of these genes during ciliogenesis of human airway epithelial cells was examined. Northern analysis demonstrated that DPCD expression increases during ciliated cell differentiation; the expression of POLL decreases. To examine directly whether DPCD is mutated in cases of human PCD, the complete coding sequence of DPCD was sequenced from 51 unrelated PCD patients. No disease-causing mutations were confirmed; however, one variant could not be excluded. Therefore, DPCD remains a novel candidate gene for PCD.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/2/174

Alveolar type II cells increase lipogenesis and convert glycogen into the phospholipids of surfactant in the late term fetal lung. Recent studies suggest that CCAAT/enhancing-binding protein (C/EBP) isoforms and sterol regulatory element binding protein (SREBP)-1c regulate fatty acid synthesis in adult type II cells in vitro. To define the temporal relationships and enzymes involved in lipogenesis in fetal rat lung, the mRNA levels of selected transcription factors and enzymes were determined. There was an increase in the mRNA levels of C/EBP(alpha), C/EBP(beta), C/EBP(delta), peroxisomal proliferator-activated receptor (gamma) (PPAR(gamma)), and SREBP-1c, but not SREBP-1a or SREBP-2 from fetal Days 19-21. There was also an increase in the mRNA levels of fatty acid synthase, stearoyl-CoA desaturase 1 (SCD-1), fatty acid translocase, glycerol-3-P acyl transferase, and phosphatidate cytidylyltransferase. By in situ hybridization, there was detectible expression of fatty acid synthase, SCD-1, and C/EBP(alpha) along the alveolar septae with the same distribution pattern as surfactant protein-C, whereas PPAR(gamma) expression appeared to be restricted to macrophages. Regulation of lipogenesis at the mRNA level is predominately on enzymes of fatty acid synthesis and appears to be regulated by C/EBP(alpha) and SREBP-1c. SCD-1 and phosphatidate cytidylyltransferase are important components of the lipogenic response in the fetal lung that have not been recognized previously.

Am. J. Respir. Crit. Care Med. (2)

Recent evidence suggests that deficiency in the Th1 cytokine pathway may underlie the susceptibility to allergic asthma. This study examined whether (1) single-nucleotide polymorphisms exist in the promoter region of the two interleukin (IL)-12 subunit genes in patients with asthma; (2) messenger RNA and protein expressions of signal transducers and activators of transcription, IL-12, IFN-(gamma), and their receptors are altered in asthma; and (3) linkage to genes in the Th1 pathway is present in families with asthma in Iceland. The promoter regions of the IL-12 subunit genes were sequenced in 94 patients with asthma and 94 control subjects without asthma. Linkage was examined in 169 families that included over 570 patients with asthma and 950 of their unaffected relatives. The results demonstrate no evidence of linkage to microsatellite markers in close association with genes within the Th1 pathway, and no polymorphism was detected in the promoter regions of the two IL-12 subunit genes in the cohort with asthma patients. Moreover, we found no differences in the messenger RNA or protein expression signals of genes in the IL-12 pathway between the patients and control subjects. We conclude that decrease in Th1 type cytokine response is unlikely to present a primary event in asthma.


Investigators have intensively evaluated the major histocompatibility (MHC) complex for sarcoidosis susceptibility genes with the majority of reports implicating the human leukocyte antigen (HLA)-DRB1 gene. Because most studies have been performed in white and Asian populations, we sought to determine which MHC genes might be risk factors for sarcoidosis in African Americans. We genotyped six microsatellite markers spanning 11.6 megabases that overlapped the MHC region on chromosome 6p21-22 in 225 nuclear families ascertained by African American probands with a history of sarcoidosis. Using a family-based association methods approach, we performed multiallelic tests of association between each marker and sarcoidosis. A statistically significant association was detected between sarcoidosis and the DQCAR marker (p = 0.002) less than two kilobases telomeric from the HLA-DQB1 gene. Typing two additional markers in this region revealed that DQCAR-G51152 haplotypes, spanning a 38-kilobase region across the HLA-DQB1 gene, were associated with sarcoidosis on a global level (p = 0.022). Analysis of individual DQCAR and G51152 alleles showed that the DQCAR 178 (expected = 21.0; observed = 10; p = 0.0005) and G51152 217 (expected = 25.6; observed = 14; p = 0.0009) alleles were transmitted to affected offspring less often than expected; whereas the DQCAR 182 allele was transmitted more often than expected (expected = 52.6; observed = 66; p = 0.002). Our results indicate that HLA-DQB1 and not HLA-DRB1 plays an important role in sarcoidosis susceptibility in African Americans. Identification of the specific HLA-DQB1 alleles that influence sarcoidosis susceptibility in African Americans and the study of their antigenic-binding properties may reveal why African Americans suffer disproportionately from this disease.

OBJECTIVE. Our purpose was to find out whether percutaneous biopsy of hepatocellular carcinoma will cause significant dissemination of tumor into the circulation by quantitative analysis of circulating tumor DNA.

SUBJECTS AND METHODS. In this prospective study of 32 patients with suspected hepatocellular carcinoma who underwent sonographically guided liver biopsy, a peripheral venous blood sample was obtained before and 5 min after the procedure. Biopsy was performed using an 18-gauge biopsy gun. DNA was extracted from the plasma of the blood samples for methylation-specific polymerase chain reaction. Quantitative measures of the plasma tumor DNA were determined with real-time quantitative polymerase chain reaction, and the amount was expressed as a methylation index (%) in plasma.

RESULTS. Nineteen (59.4%) of 32 patients did not have detectable p16 tumor suppressor gene marker (p16M) in plasma before biopsy, and they showed no detectable plasma p16M after biopsy. Thirteen (65%) of 20 patients had p16M identified in the plasma before liver biopsy. Quantitative analysis of the plasma tumor DNA in these 13 patients showed no statistically significant difference in the methylation index before and after biopsy (p = 0.345, Wilcoxon's signed rank test).

CONCLUSION. No evidence exists that percutaneous liver biopsy results in hematogenous dissemination of hepatocellular carcinoma as shown by quantitative analysis of circulating tumor DNA (p16M) using methylation-specific real-time polymerase chain reaction.


Enteroviruses (EVs), especially group B coxsackie viruses, have been implicated in the pathogenesis of myocarditis and dilated cardiomyopathy (DCM). To determine whether a specific type of EV is present in DCM hearts, we examined the genotypes of EVs detected in endomyocardial biopsies and pericardial effusions by polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis. Positive PCR results were obtained from biopsies in 6 (19%) of 31 patients with DCM, 5 (18%) of 28 with myocarditis, 5 (22%) of 23 with other cardiac diseases, and from pericardial effusions in 4 (57%) of 7 patients with pericarditis. SSCP profiles of most of the clinical samples were different and were not identical to any of the standard group B coxsackie viruses. Our findings suggest that EV genomes are involved in the myocardium of patients with various cardiac conditions and that a particular type of EV is not present in DCM hearts.


The dopamine receptor type 1 (DRD1) has been implicated in the development of hypertension in humans as well as in animal models of spontaneous hypertension. We screened the entire coding and promoter region of the human DRD1 receptor for polymorphisms to analyze their association with hypertension. The allele frequencies of two common single-nucleotide polymorphisms, A-48G and G-94A were determined in 493 hypertensive patients and 209 normotensive controls. Allele frequencies did not differ for both polymorphisms between the two groups (-48 G-allele in hypertension = 0.37; -48 G-allele in normotension = 0.36; -94 A-allele in hypertension = 0.14; -94 A-allele in normotension = 0.10). Our findings in these Caucasian patients are in contrast to a recent Japanese study that revealed a significant association of the -48 G-allele with hypertension. Thus, racial differences may play an important role concerning the association of variants in the dopamine receptor type 1 gene with essential hypertension.


Endothelial nitric oxide synthase (eNOS), encoded by NOS3, is a potent regulator of vasomotor tone and peripheral resistance. Congenic experiments indicate that a chromosomal segment containing the rat eNOS gene contributes to rat spontaneous hypertension (HT). A role for NOS3 in onset of essential hypertension (HT) is, however, controversial. We therefore decided to test NOS3 polymorphisms in a set of patients who have an accentuated ability to show an existing genetic association. The 112 HT subjects had two HT parents and the normotensive (NT) subjects had two NT parents. All were Anglo-Celtic whites. The two most promising polymorphisms, viz, a biallelic variable number of tandem repeats (VNTR) in intron 4 and an exon 7 variant that leads to an amino acid change (Glu296Asp), were genotyped by PCR (and BanII
digestion in the case of the latter). Frequency of the minor allele of the VNTR was 0.11 in the NT and 0.10 in the HT subjects (P = .9). For the exon 7 variant, Asp298 frequency was 0.30 and 0.32 in each respective group (P = .6). Tracking was seen for the Asp298 allele with elevation in body mass index (P = .034), and the minor allele of the VNTR with elevation in LDL (P = .007) and reduction in HDL (P = .048). In conclusion, we saw no association of NOS3 markers with HT in the population studied. However, possible genotypic effects on plasma lipids and body mass index might warrant further studies, especially in view of possible associations with heart disease.


[beta]-2-Adrenergic receptors ([beta]-2-AR) contribute to cardiovascular regulation by influencing several functions and previous studies suggest that a decreased function of the [beta]-2-AR may be involved in essential hypertension. [beta]-2-AR are polymorphic and certain polymorphisms of these receptors are of functional importance. We focus here on the Arg16->Gly16 [beta]-2-AR polymorphism, which shows enhanced agonist-promoted downregulation of the receptor and which, in two recent studies, yielded opposite results in terms of association with essential hypertension: an increased frequency of the Gly16 variant in African-Caribbean hypertensives and of the Arg16 variant in offspring of Norwegian white hypertensive parents. In the current study, we genotyped 243 subjects, including both African-American and white individuals, for codon 16 polymorphism and assessed blood pressure and cardiovascular function using impedance cardiography and pressor sensitivity to phenylephrine. We found similar patterns of cardiovascular function and expression of hypertension with the two genotypes of codon 16. There was no statistically significant difference in the overall allelic distribution of the two genotypes: among African-Americans, 51% of the hypertensives and 50% of the normotensives carried the Arg16 allele, whereas among the white subjects 40% of the hypertensives and 47% of the normotensives were carriers of the Arg16 allele. Although we observed a statistically significant increase in the Arg16/Gly16 heterozygotes in the African-American population, the Gly16 allele was not significantly increased in the African-Americans compared to whites. These findings indicate that the codon 16 polymorphisms are not associated with hypertension in a mixed American study population nor do they appear to substantially impact on a variety of hemodynamic variables.


BackgroundOur aim was to determine whether the aldosterone synthase (CYP11B2) -344 C/T polymorphism was associated with the blood pressure (BP)-lowering response to antihypertensive treatment. Methods Patients with mild-to-moderate primary hypertension and left ventricular hypertrophy were randomized in a double-blind study to receive treatment with either the angiotensin II type 1 (AT1) receptor antagonist irbesartan (n = 43), or the [beta]1-adrenergic receptor blocker atenolol (n = 43). The aldosterone synthase (CYP11B2) -344 C/T polymorphism was analyzed using solid-phase minisequencing and related to BP reduction after 3 months.
treatment. Serum aldosterone levels were measured.

Results

After 3 months treatment the mean reductions in BP were similar for both treatment groups. When assessing the systolic BP reduction in the irbesartan group, patients with the TT variant had a more pronounced reduction (-21 +/- 19 SD mm Hg, n = 17) than both the TC (-14 +/- 18 mm Hg, n = 18) and CC (0 +/- 17 mm Hg, n = 8) genotypes (P =.04). There was no association between this polymorphism and the diastolic BP response. The -344 C/T polymorphism was not associated with the BP response to atenolol. Nor was it related to the baseline serum aldosterone level.

Conclusions

The aldosterone synthase -344 C/T polymorphism was related to the BP-lowering response in hypertensive patients treated with the AT1-receptor antagonist irbesartan.


http://www.sciencedirect.com/science/article/B6T0Y-44M2WRR-8/2/fa7e1c75c05da71f4892ed4950e39e

[beta]2-Adrenoceptor ([beta]2-ADR)-mediated vasodilatation decreases vascular reactivity and blood pressure (BP) and chromosome 5 where its gene (ADRB2R) resides and shows linkage to hypertension (HT). A Gln27Glu ADRB2R variant confers resistance to agonist-induced desensitization and enhanced vasodilator response to isoprenaline. Therefore, we carried out a case-control study in a cohort of HT and normotensive (NT) Anglo-Celtic Australian white subjects whose parents had a similar BP status as the subjects. Glu27 frequency was 0.41 in 108 HT and 0.42 in 141 NT ((chi)2 = 0.05, P =.82). Within the HT group, the Glu27 allele was more prevalent in 61 subjects who were overweight (body mass index [BMI] >= 25 kg/m2) compared with 41 who were lean (BMI 2); ie, 0.49 v 0.31, respectively ((chi)2 = 6.4, P =.012). Furthermore, Glu27 tracked with elevation in BMI in these subjects: 24 +/- 4 kg/m2, 27 +/- 5 kg/m2, and 28 +/- 5 kg/m2 for Gln/Gln, Gln/Glu, and Glu/Glu, respectively (P =.0058 by one-way ANOVA). Thus, the Gln27Glu [beta]2-ADR variant is excluded in HT, but might influence body weight.


http://www.sciencedirect.com/science/article/B6T0Y-4937S58-F/2/715c546f37a4084c124e595efa6a3fa0

In human hypertension (HT) plasma tumor necrosis factor (TNF-[alpha]) and soluble TNF receptor 2 fragment (sTNF-R2) are increased, and the TNF-R2 gene (TNFRSF1B) has been implicated. Therefore, we measured Tnfr2 mRNA in kidney, adrenal, heart, and aorta from rats with ACTH-induced, corticosterone-induced, and spontaneous HT (SHR), and tested the effect of blockade of TNF-[alpha] by a recombinant TNF-R2 fragment (huTNFR:Fc) on development of HT in the ACTH model. Tnfr2 mRNA was quantified by real-time polymerase chain reaction, as were internal controls, [beta]-actin, and glyceraldehyde-3-phosphate dehydrogenase mRNA. The results showed no differences in tissue Tnfr2 mRNA between HT and control rats. The ACTH-induced HT was not affected by huTNFR:Fc coadministration. The findings thus offer no support for altered Tnfr2 expression in the rat models studied.

To evaluate the effects of nongenetic factors, aging, and salt-loading on the quantitative trait loci (QTLs) for blood pressure (BP), we conducted a genome-wide linkage analysis using multiple sets of BP measurements in 125 male F2 generation cross derived from stroke-prone spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. The experiment was arranged in two stages. In the first stage, corresponding to the developing period of the rats, BP was measured repeatedly without loading of salt; this continued until the rats were 5 months of age. In the second stage, after the baseline BP leveled off, 1% salt water was given to the rats and BP was monitored for the subsequent 7 months. Genome scanning was performed using 201 markers. In the developing period, three QTLs were identified on chromosomes 1, 3, and 4 (logarithmic odds [LOD] scores of 5.6, 3.1, and 3.2, respectively), which had peaks at 8 or 10 weeks of age. In the latter salt-loading stage, QTLs for BP were detected on chromosomes 1 and 10 (LOD scores 4.6 and 4.5, respectively). When the BP increase during salt-loading was analyzed as a phenotype, however, only the region on chromosome 10 showed linkage at a suggestive level (LOD score 3.2). The present study provides experimental evidence that QTLs for BP could be modulated by nongenetic factors, such as aging and salt-loading.


Background Earlier studies from this laboratory showed that central angiotensin II (AngII) receptors are upregulated by chronic cold exposure. The purpose of this study was to determine whether central AngII receptors may play a role in the development of cold-induced hypertension. Methods Four groups of rats (six rats each) were used. Two groups were exposed to cold (5\[deg\]C) and the other two groups were kept at 25\[deg\]C. One cold-exposed and one warm-adapted group were treated chronically, via osmotic minipumps, with AngII type 1 (AT1) receptor blocker (losartan, 6.0 [mu]g/2.5 [mu]L/h, intracerebroventricularly) at the beginning of cold exposure. Results Systolic blood pressure (BP) of the cold-exposed untreated group increased during week 1 of cold exposure and rose to 160 +/- 4 mm Hg by week 4, whereas BP of the losartan-treated group in cold did not increase and remained at 121 +/- 3 mm Hg. Cold-induced increases in drinking response to AngII, plasma renin activity, and urine norepinephrine output disappeared in the treated rats, indicating blockade of central AngII receptors. Withdrawal of losartan at 4 weeks resulted in an increase in BP of this group to the cold-exposed untreated level, which was accompanied by an increase in the above parameters. Significant increases in AngII-induced drinking response and hypothalamic AT1 receptor mRNA content of the cold-exposed rats indicate upregulation of AngII receptors during chronic cold exposure. Hypothalamic AngII level was not affected by cold exposure. Conclusion Upregulation of brain AT1 receptors plays a role in the development of cold-induced hypertension.

the etiology of essential hypertension (HT) in a study involving southern European whites. We attempted to replicate this finding in a well-characterized, extensively studied group of 112 white Australians with essential HT, with strong family history (two HT parents), early-onset, moderate to severe disease, and of British extraction. Controls were 196 normotensive (NT) white subjects whose parents were both NT older than age 50 years. A mismatch polymerase chain reaction method involving BanII was developed for genotyping. Frequency of the Trp460 allele was 0.23 in the HT and 0.24 in the NT groups ($\chi^2 = 0.2$, $P = .7$). No association was observed with blood pressure, body mass index, age, plasma renin, angiotensinogen, angiotensin converting enzyme, cholesterol, triglycerides, or HDL or LDL cholesterol. Our results therefore provide no support for a role for the [alpha]-adducin variant in hypertension, at least in our severely affected Anglo-white group with strong family history of HT.

American Journal of Obstetrics and Gynecology (5)


Objective

Our purpose was to determine the presence of [alpha]1-adrenoceptor messenger RNA subtypes and extend the pharmacologic characterization of [alpha]1-adrenoceptors involved in human umbilical vein (HUV) contraction.

Study design

Cords (n = 124) from healthy patients after term vaginal or cesarean deliveries were used. The vein was carefully dissected out of cords and used for reverse transcription combined with polymerase chain reaction (RT-PCR) to amplify [alpha]1-adrenoceptor transcripts. In isolated organ baths, HUV rings were mounted and cumulative concentration-response curves were constructed either for epinephrine or the selective [alpha]1A-adrenoceptor agonist, A-61603. In other series of experiments, the effects of the selective [alpha]1A- and [alpha]1B-adrenoceptor antagonists (RS-100329 or B8805-033 or spiperone, AH11110A and cyclazosin, respectively) were evaluated to estimate its blocking potencies on epinephrine concentration-response curves.

Results

By means of RT-PCR technique [alpha]1a- and [alpha]1b-adrenoceptor transcripts were detected in the HUV. The blocking potency values of RS-100329 or B8805-033 against responses mediated by epinephrine were not consistent with the activation of an [alpha]1A-adrenoceptor population. Moreover, the low potency of the agonist A-61603 was not in accordance with an [alpha]1A-adrenoceptor interaction. On the other hand, the antagonist potencies of spiperone, AH11110A and cyclazosin were in agreement with an interaction on [alpha]1B-adrenoceptor subtype.

Conclusion

Although [alpha]1a- and [alpha]1b-adrenoceptor messenger RNAs are detected in the HUV, only [alpha]1B-adrenoceptors are involved in epinephrine vasoconstrictor action.


http://www.sciencedirect.com/science/article/B6W9P-49M68N9-1F/2/bdf9b2e9a1781065ae538aedfc975a48
OBJECTIVE: The purpose of this study was to identify the factors that affect the quality of cytologic cervical cancer screening conducted in public sector clinics in Sonora, Mexico.

STUDY DESIGN: We assessed the quality of cervical cytology before and after a 2-hour training session and the implementation of cervical spatulas and endocervical brushes. Additionally, we conducted a cross-sectional study of reproductive aged women who attended public clinics in paired border communities in Sonora and Arizona. Cervical cytologic specimens (n = 2436) were collected and compared for adequacy and cytologic diagnosis and for the prevalence of human papillomavirus infection. RESULTS: The training intervention achieved significantly improved the rates of satisfactory but limited smears and unsatisfactory smears. The cross-sectional study revealed comparable quality indicators for cytologic specimens that were collected in Sonora and Arizona clinics. CONCLUSION: A high-quality cytology-based cervical cancer screening program is possible in public sector clinics in Sonora, with indicators similar to those achieved in comparable Arizona clinics.


http://www.sciencedirect.com/science/article/B6W9P-4CYPGKB-1H/2/903e6446b886fac735a8a9e4ec031d70

ObjectiveOvarian cancer cell lines and tissues express gonadotropin receptors. Conjugation of cytostatics to ligands of these receptors may increase the specificity of cytotoxic drugs. Study designToxicity of doxorubicin-human chorionic gonadotropin conjugates was determined in 4 ovarian cancer cell lines. Expression and regulation of luteinizing hormone/human chorionic gonadotropin receptors were analyzed before and after treatment with human chorionic gonadotropin, epidermal growth factor, and 8-bromo-cyclic adenosine monophosphate with a nested reverse transcriptase-polymerase chain reaction approach. Results Toxicity of human chorionic gonadotropin-doxorubicin conjugates was increased compared with unconjugated doxorubicin in OVCAR-3 cells. However, drug conjugates failed to demonstrate increased toxicity in other cell lines, especially after preincubation with human chorionic gonadotropin. All cell lines expressed luteinizing hormone/human chorionic gonadotropin receptors. Receptor expression in OVCAR-3 cells was not effected by human chorionic gonadotropin, endothelial growth factor, or 8-bromo-cyclic adenosine monophosphate treatment. In other cell lines, receptor expression was down-regulated by these agents. Conclusion Cytotoxic activity of doxorubicin was increased specifically by conjugation to human chorionic gonadotropin. However, the regulation of luteinizing hormone/human chorionic gonadotropin receptor expression and other compounds may reduce the drug-uptake of the conjugates.


http://www.sciencedirect.com/science/article/B6W9P-49W2CY2-23/2/fe6db556f4a4251c361e828558eb31b2

ObjectiveOur purpose was to show the effects of pre-B-cell colony-enhancing factor on the genes that are expressed by the human fetal membranes. Study design Explants of fetal membranes (amnion, chorion, and decidua) from three term patients were treated with 100 ng/mL recombinant human pre-B-cell colony-enhancing factor for 4 hours. RNAs were hybridized to gene chips that contained >18,000 known genes. One experiment was done in triplicate to assess replication. Data were analyzed to quantitate the signal intensities of each complementary
DNA on the array. Confirmation of the results was carried out on tissues from nine other patients by the measurement of the proteins or quantitative real-time reverse transcriptase-polymerase chain reaction. Results: Replication gave Confirmation. Pre-B-cell colony-enhancing factor appears to be at the proximal end of the pathway to labor initiation and may link sterile distention-induced labor with that of infection-induced labor.


OBJECTIVE: Our purpose was to determine the frequency of allele loss in the region of the BRCA1 gene in cancers of women who have both breast and ovarian cancer. STUDY DESIGN: Four polymorphic microsatellite markers on chromosome 17q11-21 were examined by the polymerase chain reaction in deoxyribonucleic acid from paraffin blocks of normal tissues, breast cancers, and ovarian cancers in 24 women who had primary cancers in both sites. RESULTS: Loss of heterozygosity was seen in one or more markers on chromosome 17q11-q21 in 46% of breast cancer and 78% of ovarian cancers. In 38% of cases allele loss was seen in both cancers, and in all these cases the same allele was lost in both cancers. Significantly younger ages at diagnosis of both breast and ovarian cancer were noted among cases with allele loss in both cancers compared with cases in which allele loss was found only in the ovarian cancer. RESULTS: Loss of heterozygosity was seen in one or more markers on chromosome 17q11-q21 in 46% of breast cancer and 78% of ovarian cancers. In 38% of cases allele loss was seen in both cancers, and in all these cases the same allele was lost in both cancers. Significantly younger ages at diagnosis of both breast and ovarian cancer were noted among cases with allele loss in both cancers compared with cases in which allele loss was found only in the ovarian cancer. CONCLUSIONS: Because cases in which 17q11-21 allele loss was seen in both cancers had a young age of onset and the same allele was always deleted in both cancers, hereditary alterations in BRCA1 may play a role in this subset. The older age of onset in cases in which allele loss was seen only in the ovarian cancer suggests that the development of these cancers is not related to an inherited defect in BRCA1.


Purpose: To report a phenotypic variant of lattice corneal dystrophy associated with two missense changes, Ala546Asp and Pro551Gln, in the transforming growth factor-β1-induced gene (TGFBI). Design: Experimental study. Methods: Genomic DNA was obtained from the proband as well as affected and unaffected family members. Exons 4, 11, 12, and 14 of the TGFBI gene were amplified and sequenced. Additionally, a corneal button excised from the proband was examined by light and transmission electron microscopy. Haplotype analysis was performed on the proband's family and members of a previously identified pedigree with the same TGFBI gene missense changes. Results: Bilateral, symmetric, radially arranged, branching refractile lines within and surrounding an area of central anterior stromal haze were noted in the proband. Multiple
polymorphic, refractile deposits were noted in the mid and posterior stroma in both the proband and her daughter. Light and electron microscopic analyses demonstrated amyloid and excluded the presence of deposits characteristic of granular corneal dystrophy. Screening of TGFBI exon 12 in the proband and her affected daughter revealed two missense changes, Ala546Asp and Pro551Gln (both absent in 250 control chromosomes). Haplotype analysis suggested that the mutations in this family and in a previously identified pedigree reflect a founder effect, rather than an independent occurrence.

Conclusions: We present a phenotypic variant of lattice corneal dystrophy associated with the Ala546Asp and Pro551Gln missense changes in exon 12 of the TGFBI gene. A common ancestor appears to account for the missense mutations observed in this pedigree and in a previously reported family.


http://www.sciencedirect.com/science/article/B6VK5-4BVK5C0-C/2/fff9d2e1e8522533dc942b4db2bda860

Purpose: To further characterize the mutations within the CHST6 gene responsible for causing macular corneal dystrophy in a cohort of affected patients from the United States.
Design: Experimental study.
Methods: Genomic DNA was extracted from buccal epithelium of 16 affected patients (14 families), 17 unaffected relatives, and 127 controls, followed by polymerase chain reaction amplification and direct sequencing of the CHST6 coding region. Subtyping of affected patients into type I and II macular corneal dystrophy was performed by measuring antigenic keratan sulfate (AgKS) serum levels. Haplotype analysis was performed in families that demonstrated common mutations.
Results: CHST6 coding region analysis in 10 patients identified as having type I macular corneal dystrophy revealed 10 sequence changes: eight missense mutations, four of which are novel (Met104Val, Tyr110Cys, Gln122Pro, and Leu276Pro) and four of which have been reported previously (Ser51Leu, Pro72Ser, Cys102Gly, and Leu200Arg); one novel homozygous nonsense mutation in two patients from a single family (c.1683C>T, Gln331X); and one frameshift mutation in a heterozygous state in a single patient (c.1744_1751dupGTGCAGCTG). Mutation analysis in the four patients identified as having type II macular corneal dystrophy (serum samples were not obtained from two affected patients) revealed three patients heterozygous for either the c.923G>C, c.969C>A, or c.1519T>C sequence changes. The fourth patient was compound heterozygous for c.969C>A and c.1291T>G. None of these changes was observed in 127 control individuals. Haplotype analysis using microsatellite markers flanking the CHST6 gene did not reveal a common founder for the Leu200Arg (1291T>G) missense mutation, present in five families, identifying this position as a mutation hot-spot.
Conclusions: A variety of previously unreported mutations in the coding region of the CHST6 gene are associated with type I macular corneal dystrophy in a cohort of patients from the United States.


http://www.sciencedirect.com/science/article/B6VK5-4343B5NF/2/f3a4878e85186ada130e682fcba4140

Purpose: The inheritance of specific apolipoprotein E alleles has been linked to atherosclerosis, Alzheimer disease, and, most recently, to the incidence of age-related macular degeneration. Apolipoprotein E is a common component of the extracellular plaques and deposits...
characteristic of these disorders, including drusen, which are a hallmark of age-related macular degeneration. Accordingly, we assessed the potential biosynthetic contribution of local ocular cell types to the apolipoprotein E found in drusen.

METHODS: We measured apolipoprotein E mRNA levels in human donor tissues using a quantitative assay of apolipoprotein E transcription, and we localized apolipoprotein E protein to specific cell types and compartments in the neural retina, retinal pigmented epithelium, and choroid using laser scanning confocal immunofluorescence microscopy. RESULTS: Apolipoprotein E immunoreactivity is associated with photoreceptor outer segments, the retinal ganglion cell layer, the retinal pigmented epithelium basal cytoplasm and basal lamina, and with both collagenous layers of Bruch membrane. Apolipoprotein E appears to be a ubiquitous component of drusen, irrespective of clinical phenotype. It also accumulates in the cytoplasm of a subpopulation of retinal pigmented epithelial cells, many of which overlie or flank drusen. Mean levels of apolipoprotein E mRNA in the adult human retina are 45% and 150% of the levels measured in liver and adult brain, the two most abundant biosynthetic sources of apolipoprotein E. Apolipoprotein E mRNA levels are highest in the inner retina, and lowest in the outer retina where photoreceptors predominate. Significant levels of apolipoprotein E mRNA are also present in the retinal pigmented epithelium/choroid complex and in cultured human retinal pigmented epithelial cells. CONCLUSIONS: Apolipoprotein E protein is strategically located at the same anatomic locus where drusen are situated, and the retinal pigmented epithelium is the most likely local biosynthetic source of apolipoprotein E at that location. Age-related alteration of lipoprotein biosynthesis and/or processing at the level of the retinal pigmented epithelium and/or Bruch membrane may be a significant contributing factor in drusen formation and age-related macular degeneration pathogenesis.


http://www.sciencedirect.com/science/article/B6VK5-3Y2N7W7-3/2/8f87621b6a96311333288cd81343836e

PURPOSE: Meesmann corneal dystrophy is an autosomal dominant disorder characterized by fragility of the anterior corneal epithelium. We have previously demonstrated that this disease can be caused by mutations in the genes encoding keratins K3 or K12, the major intermediate filament proteins expressed in corneal epithelial cells. Here, we have carried out mutation analysis in a United States kindred presenting with typical features of Meesmann corneal dystrophy.

METHODS: Exons 1 and 6 of the K12 gene (KRT12) were polymerase chain reaction amplified from the proband's and control DNA and subjected to direct automated sequencing. RESULTS: A heterozygous missense mutation 1300A->G was detected in exon 6 of KRT12, predicting amino acid substitution I426V in the helix termination motif of the K12 polypeptide. The mutation was confirmed in the proband and excluded from 50 normal individuals by restriction enzyme analysis of polymerase chain reaction products. CONCLUSION: We report a novel mutation in a critical molecular overlap region of K12 in a United States family with Meesmann corneal dystrophy. The results confirm that mutations in the corneal keratins (K3 or K12) can underlie Meesmann corneal dystrophy.


http://www.sciencedirect.com/science/article/B6VK5-42G0MJX-C/2/6555ab38842b29a386b1e95a9a380e6c

PURPOSE: To search for patients with Usher syndrome type IC among those with Usher
syndrome type I who reside in New England. METHODS: Genotype analysis of microsatellite markers closely linked to the USH1C locus was done using the polymerase chain reaction. We compared the haplotype of our patients who were homozygous in the USH1C region with the haplotypes found in previously reported USH1C Acadian families who reside in southwestern Louisiana and from a single family residing in Lebanon. RESULTS: Of 46 unrelated cases of Usher syndrome type I residing in New England, two were homozygous at genetic markers in the USH1C region. Of these, one carried the Acadian USH1C haplotype and had Acadian ancestors (that is, from Nova Scotia) who did not participate in the 1755 migration of Acadians to Louisiana. The second family had a haplotype that proved to be the same as that of a family with USH1C residing in Lebanon. Each of the two families had haplotypes distinct from the other. CONCLUSION: This is the first report that some patients residing in New England have Usher syndrome type IC. Patients with Usher syndrome type IC can have the Acadian haplotype or the Lebanese haplotype compatible with the idea that at least two independently arising pathogenic mutations have occurred in the yet-to-be identified USH1C gene.


http://www.sciencedirect.com/science/article/B6VK5-47DKVG-4/2/113e2d2a6a314973ea44700d4a0e70

Purpose: To determine whether cytomegalovirus (CMV) retinitis that responded poorly to intravenous ganciclovir therapy but responded to the ganciclovir implant was caused by virus with resistance mutations in the viral UL97 and UL54 genes. Design: Retrospective chart review and laboratory-based experimental study. Methods: Regions of the CMV UL97 and UL54 were amplified from the vitreous and analyzed for resistant mutations by a combination of DNA sequencing and restriction digestion. Vitreous from patients with AIDS and retinal infections other than CMV retinitis served as negative controls. Results: We amplified all target regions of UL97 DNA and most target regions of UL54 DNA from eight eyes with CMV retinitis. In one eye we found a ganciclovir resistance mutation at base 1781 of the UL97 gene, predicting an alanine to valine mutation at codon 594. In a second eye we found a ganciclovir resistance mutation at base 2960 of the UL54 gene, predicting an alanine to glycine mutation at codon 987. In two additional eyes, both from patients with bilateral retinitis, we found UL54 mutations that are likely to confer resistance to ganciclovir but have not been previously described. In both of these patients the UL54 genotype differed between the two diseased eyes. Conclusions: Failure to control CMV retinitis with intravenous ganciclovir does not necessarily imply infection with a virus having a known mutation that confers drug resistance. The ganciclovir implant can be an effective therapy for CMV retinitis caused by virus with certain UL97 and UL54 resistance mutations. Cytomegalovirus UL54 genotypes can differ between eyes in patients with bilateral retinitis.


http://www.sciencedirect.com/science/article/B6VK5-44R37H5-R/2/af9ceb60014ae4963c9478848262adb
polymerase chain reaction using two sets of universal eubacterial primers in triplicate with untreated Taq, Taq treated with 8-methoxypsoralen with ultraviolet irradiation, and Taq treated with Sau 3A1. RESULTS: Using untreated Taq, false-positive results were obtained in nested polymerase chain reaction with all 10 control samples, which were not seen with the other two methods of nested polymerase chain reaction. However, the sensitivity of nested polymerase chain reaction using Sau 3A1 was the same sensitivity as the conventional culture (34.4%), whereas the sensitivity of the nested polymerase chain reaction using 8-methoxypsoralen was 46.9% higher than in the conventional culture. CONCLUSION: To eliminate the problem of false positives in bacterial nested polymerase chain reaction, we recommend the routine utilization of Taq treated with 8- methoxypsoralen and ultraviolet irradiation.


http://www.sciencedirect.com/science/article/B6VK5-417WDMG-1P/2/73452d192c76856d7cda501d5fa88571

PURPOSE: To screen a population with primary open-angle glaucoma for mutations in the gene that encodes the trabecular meshwork inducible glucocorticoid response protein (TIGR), also known as myocilin (MYOC). METHODS: Ophthalmologic information was collected for study subjects with primary open-angle glaucoma and their relatives. Mutation screening of 74 primary open-angle glaucoma probands was conducted by sequencing TIGR/MYOC coding sequence and splice sites. RESULTS: In 23 families we detected 13 nonsynonymous sequence changes, nine of which appear to be mutations likely to cause or contribute to primary open-angle glaucoma. Two mutations, Arg272Gly and Ile499Ser, and one nonsynonymous sequence variant, Asn57Asp, are novel. We found mutations in nine of 25 juvenile glaucoma probands (36%) and two of 49 adult-onset glaucoma probands (4%). Age classification of families rather than individual probands revealed mutations in three of nine families with strictly juvenile primary open-angle glaucoma (33%), and no mutations in 39 families with strictly adult-onset primary open-angle glaucoma (0%). In families with mixed-onset primary open-angle glaucoma containing both juvenile primary open-angle glaucoma and adult-onset primary open-angle glaucoma cases, we found mutations in eight of 26 families (31%). CONCLUSIONS: Our data suggest that Gly252Arg, Arg272Gly, Glu323Lys, Gln368STOP, Pro370Leu, Thr377Met, Val426Phe, Ile477Asn, and Ile499Ser are likely to play roles that cause or contribute to the etiology of autosomal dominant primary open-angle glaucoma. Our finding of more TIGR/MYOC mutations in families with mixed-onset primary open-angle glaucoma than in the families with strictly adult-onset primary open-angle glaucoma implies that the presence of relatives with juvenile primary open-angle glaucoma in a family could be used as a basis for identifying a subset of the population with adult-onset primary open-angle glaucoma with higher prevalence of TIGR/MYOC mutations. To address this issue, and to refine estimations of mutation prevalence in these age-defined subpopulations, prospective study of a larger population ascertained entirely through adult-onset primary open-angle glaucoma probands will be needed.


http://www.sciencedirect.com/science/article/B6VK5-49W1YF4-D/2/a0c7d171789b5801d67b4d08795e73ec

PurposeTo describe an American family with lattice corneal dystrophy type I, which associates with a novel mutation, Leu569Arg, of the TGFBI (BIGH3) gene. Design Experimental
study. Methods Genomic DNA was extracted from buccal epithelial cells of four affected members of an American family with lattice corneal dystrophy type I. All 17 exons of the TGFBI gene were evaluated by PCR amplification and direct sequencing. Clinical and histologic data were also collected. Results Three generations of this family have been positively diagnosed with lattice corneal dystrophy, indicating autosomal dominant inheritance. We identified a heterozygous point mutation that associates with the disease phenotype. The single base-pair substitution (T1753G) results in an amino acid substitution (Leu569Arg) in exon 13 of the TGFBI gene. Conclusions Substitution of arginine for leucine at position 569 of the TGFBI gene results in a form of lattice corneal dystrophy that is phenotypically similar to other genetically distinct forms of type I disease. This is the first report of disease correlated with changes in exon 13 of the TGFBI gene.

Anaerobe (3)


http://www.sciencedirect.com/science/article/B6W9T-4DD8F07-1/2/d94f8df08d315a30d3d4d77f459020662

The kinetics of botulinum toxin gene expression have been investigated in Clostridium botulinum type A strains 62A, Hall A-hyper, and type A(B) strain NCTC 2916 during the growth cycle. The analyses were performed in TPGY and type A Toxin Production Media (TPM). The mRNA transcript levels encoding the proteins of the neurotoxin complex were determined using Northern analyses. Neurotoxin concentrations in culture supernatants and lysed cell pellets were assayed using ELISA, Western blots, and mouse bioassay. Proteolytic activation of botulinum neurotoxin during the growth cycle was evaluated by Western blots. For all three strains, mRNA transcripts for the toxin complex genes were initially detected in early log phase, reached peak levels in early stationary phase, and rapidly decreased in mid-to-late stationary phase and during lysis. Toxin expression varied depending on the strain and growth medium. Toxin production was highest in strain Hall A-hyper, followed by NCTC 2916 and 62A. For C. botulinum strain Hall A-hyper, cell lysis and toxin release into the supernatant occurred rapidly for cells grown in TPM, while cells grown in TPGY remained in stationary phase with minimal lysis and toxin release through 96 h of growth. In contrast, strains 62A and NCTC 2916 lysed more extensively than Hall A-hyper in TPGY. TPM supported higher toxin production and activation than TPGY in strains 62A and Hall A-hyper. These data support that the genes of the botulinum neurotoxin complex are temporally expressed during late-log and early stationary phase and that toxin complex formation depends on the strain and growth medium. Botulinum toxin synthesis and activation appears to be a complex process that is highly regulated by nutritional and environmental conditions. Further research is needed to elucidate the sensing mechanisms and genetic regulatory factors controlling these processes.


http://www.sciencedirect.com/science/article/B6W9T-48VWDSG-7/2/9bb8c6c7da2e86f805b029c00f86d5fa
Colonic spirochetosis is an inflammatory bowel disease that affects a broad range of hosts, including human and non-human primates. The disease in humans and non-human primates is characterized by intimate attachment of the anaerobic spirochetes Brachyspira aalborgi and B. pilosicoli, and some unclassified flagellated microbes along the apical membrane of colonic enterocytes. Although the presence of spiral-shaped bacteria with single polar flagella and blunted ends in colonic spirochetosis is well established, the identities of many of these organisms is still unknown. Recently, Helicobacter species with a morphology similar to the flagellated bacteria present in colonic spirochetosis have been cultured from intestinal specimens obtained from rhesus macaques, some with idiopathic colitis. The purpose of the present study was to determine whether or not the flagellated bacteria seen in the colons of rhesus macaques with colonic spirochetosis are Helicobacter. The presence of flagellated bacteria alone (n=2) or together with spirochetes (n=1) in formalin-fixed and paraffin-embedded colons of three rhesus macaques with the naturally occurring disease was demonstrated by immunohistochemical staining and ultrastructural examination. Total DNA extracted from affected and control intestinal specimens was amplified by polymerase chain reaction (PCR) using Helicobacter 16S rRNA gene-specific primers. Comparative nucleotide sequence analysis of PCR products cloned from positive reactions indicated that two distinct Helicobacter genomospecies were present either alone or in combination with Brachyspira in the colons of rhesus macaques with microscopic lesions indicative of colonic spirochetosis.

Rafii, F. and M. Park "Effects of gyrase mutation on the growth kinetics of ciprofloxacin-resistant strains of Clostridium perfringens." Anaerobe In Press, Corrected Proof  
http://www.sciencedirect.com/science/article/B6W9T-4FM5D5P-1/2/08ed773e354cd6d3e9a240a323a6a49

To investigate the effect of gyrA mutation on resistance of Clostridium perfringens to fluoroquinolones, a ciprofloxacin-resistant mutant was developed. The mutant had a single substitution in gyrA at position 87 (Asp to Tyr) and no additional mutations in gyrB, parC or parE. The MIC values of gatifloxacin and ciprofloxacin for this strain were 16 and 32-fold higher than those for the wild type, which were 0.125 and 0.250 [mu]g/mL, respectively. The resistant mutant grew equally well in the presence or absence of 5 [mu]g/mL of ciprofloxacin or 1 [mu]g/mL of gatifloxacin and grew to lower cell densities with up to 30 [mu]g/mL of ciprofloxacin or 5 [mu]g/mL of gatifloxacin. Higher concentrations of fluoroquinolones resulted in increases in the time required to reach the end of the exponential phase and in lower cell densities at the end. The efflux pump inhibitor reserpine did not affect susceptibility to fluoroquinolones. The substitution of Asp 87 to Tyr in gyrA may have protected C. perfringens from low concentrations of ciprofloxacin and gatifloxacin and enabled survival and growth at higher concentrations.

Analytica Chimica Acta (5)

http://www.sciencedirect.com/science/article/B6TF4-44724KH-B/2/832d81d728b4a21b09327adc11d3f0ce
A highly sensitive single-stranded conformation polymorphism method for analysis of Vitamin D receptor (VDR) gene polymorphism was developed employing laser-induced fluorescence capillary electrophoresis (LIF-CE). LIF-CE was conducted utilizing a linear polyacrylamide solution as entangled polymer and acridine orange as a fluorescent dye of single-stranded DNA. Effect of acridine orange, size of PCR product and running temperature were investigated by LIF-CE in order to analyze the two polymorphisms of the allelic variation of the Bsm I and Taq I sites in intron 8 and exon 9, respectively, of the VDR gene. The developed method was simple, rapid (Taq I type, Bsm I type) and highly sensitive for VDR gene polymorphism. VDR gene polymorphism in 32 subjects was determined by the proposed method. The results were consistent with those obtained by restriction fragment-length polymorphism (RFLP) analysis using gel electrophoresis. The proposed method can be employed among the various VDR gene polymorphism analyses related to osteoporosis.


http://www.sciencedirect.com/science/article/B6TF4-447DB51-1/2/7ab9262ef106be0ecd7053b098e02347

There is an increasing need for field-portable systems for the detection and characterization of microorganisms in the environment. Nucleic acids analysis is frequently the method of choice for discriminating between bacteria in complex systems, but standard protocols are difficult to automate and current microfluidic devices are not configured specifically for environmental sample analysis. In this report, we describe the development of an integrated DNA purification and polymerase chain reaction (PCR) amplification system and demonstrate its use for the automated purification and amplification of Geobacter chapellei DNA (genomic DNA or plasmid targets) from sediments. The system includes renewable separation columns for the automated capture and release of microparticle purification matrices, and can be easily reprogrammed for new separation chemistries and sample types. The DNA extraction efficiency for the automated system ranged from 3 to 25%, depending on the length and concentration of the DNA target. The system was more efficient than batch capture methods for the recovery of dilute genomic DNA even though the reagent volumes were smaller than required for the batch procedure. The automated DNA concentration and purification module was coupled to a flow-through, Peltier-controlled DNA amplification chamber, and used to successfully purify and amplify genomic and plasmid DNA from sediment extracts. Cleaning protocols were also developed to allow reuse of the integrated sample preparation system, including the flow-through PCR tube.


http://www.sciencedirect.com/science/article/B6TF4-44F7DFW-1M/2/b7418a542c1c347ee871086845458bd9

The determination of genetic characteristics in human bone and soft tissues is interest both to forensic scientists and to physical anthropologists, although for different purposes. The application of genetic typing from human remains to anthropological and forensic identification problems is briefly reviewed. DNA analyses of ancient and modern human remains by Southern blot (RFLP) and amplification (PCR) techniques are reviewed. The results of studies on the effects of exposing bone and soft tissues to different environmental conditions for defined time periods on subsequent DNA typing are presented. Bone specimens were temperatures and soil under dry and humid conditions, and a complete series of soft tissues were exposed to dry and
moist air and to salt water, for periods of 1-9 months.


Attempts, were made to obtain RNAs that can bind to monosaccharides (galactose, glucose, and mannose) by in vitro selection. As a result, an RNA library that can bind to galactose with outstanding affinity has been obtained. Furthermore, the library was able to distinguish galactose from other monosaccharides, though they have closely similar structures. After another selection in order to obtain fast binders, a selected library tightly bound to galactose was obtained but it lost selectivity to galactose. This selectivity seemed to be derived from hydrogen bond geometry with the epimeric hydroxyl groups of monosaccharides. The results show an ability of RNAs to recognize any small molecule which consists of only carbon, hydrogen and oxygen.


DNA amplification by the polymerase chain reaction (PCR) was monitored at the level of single molecules. The technique used consisted of a direct fluorescent labeling method using the PCR and measurement of fluorescence fluctuation by fluorescence correlation spectroscopy (FCS). An increasing number of target DNA molecules during amplification resulted in a decrease of the number fluctuations, and also an increase of the average diffusion time. Fluorescein-11-dUTP was incorporated into the DNA strand with a length of 4000 bp using Taq DNA polymerase. Increasing the apparent labeling density according to concentration of fluorescein-11-dUTP was evaluated from the fluorescence intensity per DNA molecule. The number of amplified DNA molecules could be detected quantitatively after 10 PCR cycles even when the initial template number was 3750 copies; however, a linear relationship between the initial template number and amplified DNA number was shown at 20 cycles in PCR.


We have developed a method to isolate RNA in high yield from adult articular cartilage. Homogenization of the articular cartilage with a freezer mill, extraction with 4 guanidinium
isothiocyanate/acid-phenol, and ultracentrifugation in cesium trifluoroacetate was found to be an effective and practical method for isolating a high yield of intact RNA from adult canine articular cartilage. The total RNA was suitable for Northern blot analysis. The mRNA that could then be isolated by oligo-dT affinity chromatography was found to be a suitable substrate for in vitro translation, for making a cDNA library, and for PCR amplification.


http://www.sciencedirect.com/science/article/B6W9V-4DN9TB1-8/2/92448a6086833759b9a2b536c1183688

RNA analysis is expected to play an increasingly important role in the area of biomolecular forensic analysis. For example, mRNA expression analysis performed on a total RNA sample isolated from a biological stain may be used to identify the nature of the tissue(s) comprising the stain. Many of the physiological stains encountered at crime scenes involve heterogeneous mixtures of different body fluids (e.g., semen and saliva, semen and vaginal secretions). Separate sampling of these mixed stains from different "geographical" locations of the stains to isolate DNA and RNA could result in a misleading estimate of the ratio of the body fluids present and, in extreme cases, even fail to detect one of the contributors. Thus, a prerequisite for the use of mRNA expression profiling in routine forensic analysis is the ability to co-extract DNA and RNA from the same stain. This article describes an optimized method that was specifically developed to co-extract mRNA and DNA from the same physiological stain and that appears to be sufficiently sensitive and robust for routine forensic use.


http://www.sciencedirect.com/science/article/B6W9V-49H1JMG-3/2/48ea900e9e657c0bc1485d7f812416

The amplification efficiencies of several polymerase chain reaction (PCR) enzymes were compared using real-time quantitative PCR with SYBR Green I detection. Amplification data collected during the exponential phase of PCR are highly reproducible, and PCR enzyme performance comparisons based upon efficiency measurements are considerably more accurate than those based on endpoint analysis. DNA polymerase efficiencies were determined under identical conditions using five different amplicon templates that varied in length or percentage GC content. Pfu- and Taq-based formulations showed similar efficiencies when amplifying shorter targets (Pfu formulations with dUTPase exhibited significantly higher efficiencies than Taq, Pfu, and other archaeal DNA polymerases. We discuss the implications of these results.


http://www.sciencedirect.com/science/article/B6W9V-4DYM9HN-2VP/2/822f7ceefbb179feb22d76f252cae6b9

The photochemically derived silver stain of nucleic acids in polyacrylamide gels originally described by Merrill et al. (1981, Science 211, 1437-1438) was modified to reduce unspecific
background staining and increase sensitivity (down to 1 pg/mm² band cross-section). Detection limits for double-stranded DNA fragments from HaeIII endonuclease digests of phage [phi]X174 were maintained despite eliminating oxidation pretreatment of fixed gels and reducing silver nitrate concentration. Preexposure to formaldehyde during silver impregnation enhanced sensitivity and the inclusion of the silver-complexing agent sodium thiosulphate in the image developer decreased background staining. Higher formaldehyde concentration during image development resulted in darker bands with good contrast. The procedure almost halves the number of steps, solutions and experimental time required and can be used for the staining of DNA fragments in polyacrylamide gels bound to a polyester backing film by controlling temperature during image development. We have applied this improved staining procedure for the routine analysis of complex DNA profiles generated by DNA amplification fingerprinting (DAF).


http://www.sciencedirect.com/science/article/B6W9V-4CSYKX5-9/2/8ca72c4c30d80734c352698909f63a5a

In this article, we describe a genotyping approach applicable to both individual and multiplexed single nucleotide polymorphism (SNP) analysis, based on a ligation detection reaction (LDR) performed directly on genomic DNA. During the ligation, the biallelic state of the SNP locus is converted into a bimarker state of ligated detector oligonucleotides. The state of the markers is then determined by a 5'-nuclease assay (TaqMan) with universal fluorescent probes. The LDR-TaqMan method was successfully applied for the genotyping of 30 SNP loci of Arabidopsis thaliana. The technology is cost-effective, needs no locus-specific optimization, requires minimal manipulations, and has very good potential for automation.


http://www.sciencedirect.com/science/article/B6W9V-4BN50SX-3/2/3cb7399c8b7d6ccc446f5 ada64440431


http://www.sciencedirect.com/science/article/B6W9V-47262DF-1/2/47b30237fb149a fbb8635037816c722

New Tn10 minitransposons were constructed to functionally map long-range transcription regulatory sequences in bacterial artificial chromosomes (BACs) and P1-derived artificial chromosomes (PACs). Each contained a wild-type loxP site but, significantly, contained no mammalian or bacterial genes and/or promoter elements within the transposed portion of DNA. In contrast to loxP transposons described previously, the new ones do not introduce transcription regulatory elements capable of interfering with those endogenous to the BAC clone in functional mapping studies. Progressive deletions from the loxP end of genomic DNA were efficiently generated using these transposons, and a series of truncations generated in a green fluorescence protein (GFP)-BAC fusion clone unambiguously identified three new long-range enhancer sequences functionally in the Nkx2-5 gene in transgenic mice. Insertions of these new transposons lacking antibiotic resistance genes into a BAC or PAC were indirectly selected by their ability to delete enough DNA from the clone so as to enable its packaging within a P1 phage head with both loxP sites intact for subsequent recovery of the large plasmid. The outcome of such an indirect mode of selection is both desirable and undesirable. First, because the screen is not antibiotic resistance marker dependent, the same transposon can be used to generate nested deletions efficiently in both BACs and PACs. Second, deletions through intrainsert recombinations unrelated to loxP/Cre also get packaged and recovered, and size analyses of the BAC/PAC vector band after NotI digestion is indispensable to identify authentic loxP/Cre deletions. The procedure nevertheless offers a potential approach to map recombinogenic sequences in BACs and PACs.


Millions of single nucleotide polymorphisms (SNPs) have been identified in recent years. This provides a great opportunity for large-scale association and population studies. However, many high-throughput SNP typing techniques require expensive and dedicated instruments, which render them out of reach for many laboratories. To meet the need of these laboratories, we here report a method that uses widely available DNA sequencer for SNP typing. This method uses a type II restriction enzyme to create extendable ends at target polymorphic sites and uses single-base extension (SBE) to discriminate alleles. In this design, a restriction site is engineered in one of the two polymerase chain reaction (PCR) primers so that the restriction endonuclease cuts immediately upstream of the targeted SNP site. The digestion of the PCR products generates a 5'-overhang structure at the targeted polymorphic site. This 5'-overhang structure then serves as a template for SBE reaction to generate allele-specific products using fluorescent dye-terminator nucleotides. Following the SBE, the allele-specific products with different sizes can be resolved by DNA sequencers. Through primer design, we can create a series of PCR products that vary in size and contain only one restriction enzyme recognition site. This allows us to load many PCR products in a single capillary/lane. This method, restriction-enzyme-mediated single-base extension, is demonstrated by typing multiple SNPs simultaneously for 44 DNA samples. By multiplexing PCR and pooling multiplexed reactions together, this method has the potential to score 50-100 SNPs/capillary/run if the sizes of PCR products are arranged at every 5-10 bases from 100 to 600 base range.


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We present modifications to polymerase chain reaction-based DNA sequence analysis which avoid the need for M13 cloning and allow one set of sequencing primers to be used for analysis of any desired DNA sequence. This procedure employs nested amplification primers including short 5′-terminal sequences suitable for the attachment of fluorescent markers or for sequencing with M13 universal and reverse sequencing primers. Our modifications provided adequate single-stranded DNA for reliable automated sequence analysis of selected Ha-ras gene regions, starting with less than 1 [mu]g of genomic DNA.


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Transcriptomic methods are widely used as an initial approach to gain a mechanistic insight into physiological and pathological processes. Because differences in gene regulation to be assessed by RNA screening methods (e.g., SAGE, Affymetrix GeneChips) can be very subtle, these techniques require stable reference genes for accurate normalization. It is widely known that housekeeping genes, which are routinely used for normalization, can vary significantly depending on the tissue, and experimental test. In this study, we aimed at identifying stable reference genes for a fibrillar A[beta]42 peptide-treated, human tau-expressing SH-SY5Y neuroblastoma cell line derived to model aspects of Alzheimer's disease in tissue culture. We selected genes exhibiting potential normalization characteristics from public databases to create a custom-made microarray allowing the identification of reference genes for low, intermediate, and abundant mRNAs. A subset of these candidates was subjected to quantitative real-time polymerase chain reaction and was analyzed with geNorm software. By doing so, we were able to identify GAPD, M-RIP, and POLR2F as stable and usable reference genes irrespective of differentiation status and A[beta]42 treatment.
Studies of gene expression from bone, cartilage, and other tissues are complicated by the fact that their RNA, collected and pooled for analysis, often represents a wide variety of composite cells distinct in individual phenotype, age, and state of maturation. Laser capture microdissection (LCM) is a technique that allows specific cells to be isolated according to their phenotype, condition, or other marker from within such heterogeneity. As a result, this approach can yield RNA that is particular to a subset of cells comprising the total cell population of the tissue. This study reports the application of LCM to the gene expression analysis of the cartilaginous epiphyseal growth plate of normal newborn mice. The methodology utilized for this purpose has been coupled with real-time quantitative reverse transcriptase-polymerase chain reaction (QRT-PCR) to quantify the expression of certain genes involved in growth plate development and calcification. In this paper, the approaches used for isolating and purifying RNA from phenotypically specific chondrocyte populations of the murine growth plate are detailed and illustrate and compare both qualitative and quantitative RT-PCR results. The technique will hopefully serve as a guide for the further analysis of this and other connective tissues by LCM and RT-PCR.


Deoxycytidine kinase (dCK) is necessary for the activity of several nucleosides used for the chemotherapy of cancer and AIDS. However, the measurement of dCK catalytic activity in crude cell extracts may be imprecise, due to the presence of phosphatases and nucleotidases that degrade the enzyme products. We describe a simple immunoassay for dCK that can measure accurately as little as 5 ng enzyme protein in crude tissue extracts. The assay enabled us to show (i) that mutant cells deficient in dCK activity lack immunoreactive dCK protein, (ii) that dCK catalytic activity and immunoreactivity correlate closely in human tumors, and (iii) that immunoreactive dCK is particularly high in lymphocytes and lymphoid malignancies, although certain solid tumors may also contain the enzyme. The immunoassay of dCK could prove useful in the selection and monitoring of patients who are being treated with nucleosides that are activated by this enzyme.


We developed a quantitative assay for human immunodeficiency virus type 1 (HIV-1) proviral DNA sequences using the polymerase chain reaction (PCR). The relative copy numbers of HIV-1 proviral DNA molecules were determined by coamplification of an HIV-1 gag sequence and a portion of the DQ [alpha] locus of the histocompatibility (HLA) region. Because of the disparity in the copy number of cellular and HIV-1 templates, an attenuation in the efficiency of the HLA amplification was required to achieve simultaneous amplification and quantitation of both target sequences. The HIV-1 and HLA amplified products were detected by hybridization with radioactively labeled probes and the amount of probe bound to each product was determined with a radioanalytic system. Standard curves were generated by plotting the HIV-1 and HLA signals made against known copies of each target present prior to amplification. The copies of HIV-1 target relative to the number of cells in a given sample were determined by interpolation from standard curves. The procedure described here is generally applicable to the quantitation of other retroviruses.


Adaptor-tagged competitive polymerase chain reaction (ATAC-PCR) is an advanced version of quantitative competitive PCR characterized by the addition of unique adaptors to different cDNA samples. It is currently the only quantitative PCR technique that enables large-scale gene expression analysis. Multiplex application of ATAC-PCR employs seven adaptors, two or three of which are used as controls to generate a calibration curve. The characteristics of the ATAC-PCR method for large-scale data production, including any adaptor- and gene-dependent amplification biases, were evaluated by using this method to analyze the expression of 384 mouse brain genes. Short adaptors tended to amplify at higher efficiency than did long adaptors. The population of genes with a high amplification bias increased with the use of short adaptors. Subtracting the median value of all adaptor-dependent biases could reduce this bias; the majority of genes displayed a small gene-dependent bias, which facilitated reliable quantification. We modified ATAC-PCR to estimate molecular numbers of transcripts by introducing synthetic standards. This modification demonstrated that gene expression levels in mammalian cells are varied over seven orders of magnitude.


Six TaqMan real-time polymerase chain reaction (PCR) systems using minor groove binding (MGB) probes have been developed for the detection quantitation of bovine, porcine, lamb, chicken, turkey, and ostrich DNA in complex samples. Species-specific amplification was achieved by combining only two fluorogenic probes and 10 oligonucleotide primers targeting mitochondrial sequences, decreasing the cost of the assay significantly. The limits of detection ranged from 0.03 to 0.80 pg of template DNA. Analysis of experimental mixtures containing two to four different species showed the suitability of the assay for detection of more than 1% of pork, chicken, or turkey and of more than 5% of cattle or lamb. The quantitation accuracy in samples containing 10-100% of beef or pork DNA was close to 90%. The system is complemented with one additional TaqMan MGB detector based on consensus sequence segments of the nuclear 18S ribosomal RNA gene. A method to evaluate the presence of unknown eukaryotic DNA in a mixture, where data derived from the species-specific detection are compared with the experimental values obtained from the general 18S detector, is presented. This method allows the validation of the quantitative measurements, providing an internal control of the total content of PCR-amplifiable DNA in the sample. The system was tested on DNA mixtures containing different shares of up to four different species and on DNA extracted from processed commercial food samples.


Quantitative RT-PCR using LUX primers was performed to determine the expression patterns of various transcripts in samples of pluripotent, mouse P-19 stem cells. The P-19 cells were used
because they transform into neuron-like cells upon retinoic acid treatment. The expression of neural and stem cell genes, including GLUR1, GABA-B1a, NMDA1, ChAT, BDNF, nestin, BMP-2, BMP-4, and EGR1, was increased, approximately 10- to 1000-fold, during the course of differentiation from 0 to 11 days after induction with retinoic acid. A 3-fold serial dilution of in vitro-transcribed ChAT mRNA from 66 to 107 copies was discriminated by qRT-PCR using fluorogenic LUX primers. Results of quantitation using PCR utilizing dual LUX primer pairs were similar to quantitation using single LUX primers, and to results derived by using an alternate method for qRT-PCR, the 5'-nuclease probe assay. The efficiencies of PCRs using various primer sets were similar, so that a comparative CT method of quantifying relative amounts of transcripts was performed. We conclude that real-time RT-PCR using fluorogenic LUX primers is a reliable, effective alternative to present methods for quantifying several transcripts in neural stem cells.


The use of expression profiling to explore a cell's transcriptional landscape has exploded in recent years. In many cases, however, the very limited amount of starting material poses a major problem, making the amplification of the isolated RNA obligatory. The most prominent amplification method used was developed by the Eberwine lab in 1990: cDNA synthesis is started with an oligo(dT) primer containing a T7 RNA polymerase promoter. After second-strand synthesis RNA is transcribed in vitro using T7 RNA polymerase. It has been demonstrated that antisense RNA amplification not only preserves the fidelity of RNA-based microarray analysis but even improves the sensitivity. In our aim to improve the yield of in vitro transcription reactions and to facilitate the use of amplified RNA for the construction of cDNA libraries we tested a series of T7 primers with different 3' flanking sequences containing restriction sites. In addition we tested the impact of different DNA polymerases used for synthesizing the templates on the efficiency of the in vitro transcription reaction. A total of 28 different oligo(dT)-T7 promoter primers were tested. Two of them showed a dramatically increased yield of RNA from the in vitro transcription reaction. The combination of the improved second-strand synthesis with the new T7 primer increased the RNA yield 60-fold compared to the yield of standard procedures.


The interferon-[alpha] gene is a gene family of over 20 distinct genes having 80-95% homology with one another at a nucleotide level. Because of the high homology in the gene cluster, the available interferon-[alpha] gene probes can hybridize to multiple bands of different size on Southern blot analysis of restricted human genomic DNA. We used the polymerase chain reaction with the primers synthesized from Alu repetitive sequence and the conserved sequences of the interferon-[alpha] gene cluster to generate specific probes for individual interferon-[alpha] genes. The amplification products were subcloned into a plasmid vector and analyzed by DNA sequencing and Southern blotting of the restricted human placental DNA. One clone, which derived from interferon-[alpha]14 gene, produced a single 5.2-kb band in Southern blots of the HindIII-restricted human placental DNA. This stands in contrast to the 10 bands of different size that were detected with a cDNA for the interferon-[alpha]1 gene. Our results indicate that a
polymerase chain reaction-based method can be used to isolate gene-specific sequences from the interferon-[alpha] gene cluster. Since a variety of human cancers has been found to have the complete or partial deletion of the interferon-[alpha] gene cluster, the gene-specific probe generated by this method may aid in determining the breakpoints in the vicinity of the gene cluster.

http://www.sciencedirect.com/science/article/B6W9V-4F08527-5/2/e58546ddbf0a2bd1377b3cd5e11714e2

DNA-polymerase-mediated incorporation of different fluorochrome-labeled nucleotides (FdNTPs) was investigated with the goals of optimizing the high-density labeling of probes and exploring DNA sequencing strategies that rely on the controlled, sequential addition of such compounds. By systematically evaluating variables--including polymerase type, buffer conditions, and fluorochrome chemistries--a rational strategy for the sequential addition of labeled nucleotides to a DNA template was demonstrated. A simple structural model of the polymerase-DNA template complex that considered the fluorochrome moiety of the FdNTPs and the linker length also guided this strategy. Complementary results that portend the use of simple photobleaching to enable the reliable quantitation of consecutive additions are presented.

http://www.sciencedirect.com/science/article/B6W9V-4D48XPV-1/2/e66d16e76166a3ee28a3b368b5f4accf

A new method for specific detection of proteins based on fluorescence resonance energy transfer (FRET) using affinity proteins (affibodies) derived from combinatorial engineering of Staphylococcal protein A has been developed. Antiidiotypic affibody pairs were used in a homogeneous competitive binding assay, where the idiotypic, target-specific affibody was labeled with fluorescein and the antiidiotypic affibody was labeled with tetramethylrhodamine. Intermolecular FRET between the two fluorescent probes was observed in the antiidiotypic affibody complex, but upon addition of target protein the antiidiotypic affibody was displaced, which was monitored by a shift in the relative emission of the donor and acceptor fluorophores. The feasibility of the system was demonstrated by the detection of IgA and Taq DNA polymerase with high specificity, using two different antiidiotypic affibody pairs. Detection of Taq DNA polymerase in 25% human plasma was successfully carried out, demonstrating that the method can be used for analysis of proteins in samples of complex composition.

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http://www.sciencedirect.com/science/article/B6W9V-4DYN47Y-JM/2/fbea163845983df09a5688df745bd8a2

Reverse transcription-polymerase chain reaction (RT-PCR) is a gene expression assay by which messenger RNA (mRNA) production can be measured. This technique involves three steps: isolation of RNA from cells or tissues, the creation of a DNA copy of the desired message (cDNA) by viral reverse transcriptase enzymes (RT), and amplification of this DNA segment by the polymerase chain reaction (PCR) for subsequent quantitation and analysis. Here we describe a one-enzyme, one-step method combining the RT and PCR steps of conventional RT-PCR by exploiting the recently documented RT properties of Taq polymerase, the thermostable enzyme used for PCR amplification of DNA. RNA was extracted from gibbon T-cells (MLA144), reverse transcribed and amplified with oligonucleotide primers (specific for the 5' portion of a spliced interleukin-2 (IL-2) messenger RNA) by Taq polymerase. A discrete fragment of correct length for IL-2 cDNA was detected. Experiments showed that this product was RNA-dependent and specific for IL-2. This fragment was sequenced by automation employing a biotin primer-streptavidin magnetic bead protocol which confirmed its origin as processed IL-2 mRNA. The modification of the RT-PCR procedure using a thermostable enzyme speeds up reaction time and increases stringency. This method should make the diagnostic screening of cells for gene expression more efficient and practical.


http://www.sciencedirect.com/science/article/B6W9V-46RN563-Y/2/91b78b02f6e6078519687cb94e65d1c6


AmpliDet RNA is a real-time diagnostic method, the specificity of which is defined mainly by the molecular beacon (MB). MBs can be characterized according to the stability of their stem-and-loop structures and that of the probe-target duplex via the free energies accompanying their formation. By the application of thermodynamic models, we propose a prediction method for these [Delta]G0 parameters, which was compared to experimental analysis. The average absolute discrepancies for [Delta]G041 and for the melting temperatures of MB secondary
structures were 0.30±0.26 kcal/mol and 2.15±1.5 [deg]C, respectively. ΔG041 of probe-target interaction was predicted with a discrepancy of 1.2±1.0 kcal/mol. To characterize specificity, we formulated a model system with several MBs of highly similar sequence, but different lengths, and template RNAs carrying different types of mutations. We demonstrated the ability to detect a point mutation, or to tolerate one, irrespective of mismatch type. Of the nucleotide analogues tested, universal pyrimidine was found to increase MB tolerance substantially toward polymorphism. In the present study MBs were characterized under AmpliDet RNA conditions, with respect to probe stability, binding strength, and specificity, which led us to propose a design method, useful for probe design for AmpliDet RNA and adaptable to microarrays.


A simple procedure is described for the efficient deletion of large DNA sequences. The method involves a combination of oligonucleotide-directed mutagenesis in bacteriophage M13 and amplification of the mutagenized product by polymerase chain reaction. In contrast to other protocols employing polymerase chain reaction, synthesis of only one specific primer is required. The efficiency of heteroduplex formation between mutagenic primers directing large deletions and singlestranded template is discussed.


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http://www.sciencedirect.com/science/article/B6W9V-4DYM9Y1-X7/2/20e74a7e77215b82d822da718f6f8314a

Polymerase chain reaction (PCR) amplification was employed to construct a mosaic gene consisting of the propeptide region of protein S and the glutamic acid-rich domain of osteonectin. The strategy is straightforward, results in large amounts of material, and is universally applicable for the generation of protein domain chimeras. In some cases 10% dimethyl sulfoxide aided the amplification. Four base CCGC "clamp" sequences adjacent to BamHI restriction sites at the ends of the PCR products were used to enhance the ligation of products. A hybrid inverse complement oligonucleotide primer composed of sequences containing 20 nucleotides of protein S and 16 nucleotides of osteonectin was used in the first round of PCR. An additional osteonectin sequence was added to the initial amplified product by performing PCR using a second "boot-strap" primer containing 18 nucleotides of osteonectin. Primers used to amplify osteonectin encompassed the 146-amino-acid NH2-terminal half of osteonectin. The double-stranded first-round fragments of protein S-osteonectin and osteonectin were subsequently mixed together and
one elongation cycle of PCR was performed. Annealing occurred as the result of the 34-base-pair overlap region composed of osteonectin sequence. Taq polymerase was used for elongation with subsequent recombinant DNA synthesis. After elongation, external primers were added to amplify the protein S-osteonectin gene construct. The protocol we have developed allows noncoding and coding segments of DNA to be linked, GC-rich areas of DNA to be amplified, hybridization temperatures to be increased, annealing times to be reduced, and PCR of products to be subcloned.


http://www.sciencedirect.com/science/article/B6W9V-4F08527-1/2/331acc0c75b23e7457e2eecbc03689ba

Human forensic casework requires sensitive quantitation of human nuclear (nDNA), mitochondrial (mtDNA), and male Y-chromosome DNA from complex biomaterials. Although many such systems are commercially available, no system is capable of simultaneously quantifying all three targets in a single reaction. Most available methods either are not multiplex compatible or lack human specificity. Here, we report the development of a comprehensive set of human-specific, target-specific multiplex polymerase chain reaction (PCR) assays for DNA quantitation. Using TaqMan-MGB probes, our duplex qPCR for nDNA/mtDNA had a linear quantitation range of 100 ng to 1 pg, and our triplex qPCR assay for nDNA/mtDNA/male Y DNA had a linear range of 100-0.1 ng. Human specificity was demonstrated by the accurate detection of 0.05 and 5% human DNA from a complex source of starting templates. Target specificity was confirmed by the lack of cross-amplification among targets. A high-throughput alternative for human gender determination was also developed by multiplexing the male Y primer/probe set with an X-chromosome-based system. Background cross-amplification with DNA templates derived from 14 other species was negligible aside from the male Y assay which produced spurious amplifications from other nonhuman primate templates. Mainstream application of these assays will undoubtedly benefit forensic genomics.


http://www.sciencedirect.com/science/article/B6W9V-489YV15-8/2/78fbd719f2e8f2c3d92d29a66e3a5557

We have designed and evaluated four assays based upon PCR amplification of short interspersed elements (SINEs) for species-specific detection and quantitation of bovine, porcine, chicken, and ruminant DNA. The need for these types of approaches has increased drastically in response to the bovine spongiform encephalopathy epidemic. Using SYBR Green-based detection, the minimum effective quantitation levels were 0.1, 0.01, 5, and 1 pg of starting DNA template using our bovine, porcine, chicken, and ruminant species-specific SINE-based PCR assays, respectively. Background cross-amplification with DNA templates derived from 14 other species was negligible. Species specificity of the PCR amplicons was further demonstrated by the ability of the assays to accurately detect trace quantities of species-specific DNA from mixed (complex) sources. Bovine DNA was detected at 0.005% (0.5 pg), porcine DNA was detected at 0.0005% (0.05 pg), and chicken DNA was detected at 0.05% (5 pg) in a 10-ng mixture of bovine, porcine, and chicken DNA templates. We also tested six commercially purchased meat products using these assays. The SINE-based PCR methods we report here are species-specific, are highly sensitive, and will improve the detection limits for DNA sequences derived from these
species.


http://www.sciencedirect.com/science/article/B6W9V-4956698-6/2/ed68695fcbdf1b8c77f4b2f70c15c2

Human placental choriocarcinoma (JAR) cells endogenously expressing glycine transporter type 1a (GlyT1a) have been cultured in 96-well scintillating microplates to develop a homogenous screening assay for the detection of GlyT1 antagonists. In these microplates uptake of [14C]glycine was time dependent and saturable with a Michaelis-Menten constant (Km) of 27 +/- 3 [mu]M. The GlyT1 transport inhibitors sarcosine, ALX-5407, and Org-24598 were tested and shown to block [14C]glycine uptake with expected IC50 values of 37.5 +/- 4.6 [mu]M, 2.8 +/- 0.6 nM, and 6.9 +/- 0.9 nM, respectively. The [14C]glycine uptake process was sensitive to membrane Na+ gradient as blockade of membrane Na+/K+-ATPase by ouabain or Na+ exchanger by benzamil-disrupted glycine accumulation in JAR cells. Glycine influx was not affected by concentration of dimethyl sulfoxide up to 2%. The versatility of this technological approach was further confirmed by the characterization of a saturable [14C]taurine uptake in JAR cells. Taurine transport was of high affinity with a Km of 10.2 +/- 1.7 [mu]M and fully inhibited by ALX-5407 (IC50=522 +/- 83 nM). The developed assay is homogenous, rapid, versatile and amenable to automation for the discovery of new neurotransmitter transporter inhibitors.


http://www.sciencedirect.com/science/article/B6W9V-4FFN2TY-3/2/21b79250b8fd05bad120ca911561ac03

Different methods have been developed for single nucleotide polymorphism (SNP) typing during recent years. Allele-specific polymerase chain reaction (ASPCR) is a cost-saving method that scores SNPs by difference of the PCR efficiency of allele-specific primers. However, ASPCR for SNP typing is notoriously confounded for its locus-specific unpredictability and the laborious gel electrophoresis. In the current study, we investigated the real-time kinetics of ASPCR and found that a simple touchdown thermocycling protocol improved its specificity significantly. Combined with real-time PCR, we developed a homogeneous genotyping method and scored more than 1000 genotypes, including all transition and transversion SNPs. A clear genotyping result was identified and validated the robustness of the method. Optimization of reactions and intrinsic modification of allele-specific primers, a laborious process but one that is repeatedly reported to be inevitable for successful ASPCR, was proved to be unnecessary with our method. Accuracy was confirmed with mass spectrometry. These characters enabled real-time ASPCR with the touchdown thermocycling protocol being very competitive among various SNP typing methods for large-scale genetic studies.


http://www.sciencedirect.com/science/article/B6W9V-488NV3H-
There is considerable interindividual variation in man's ability to metabolize drugs and foreign compounds. These differences can partly be attributed to genetic polymorphisms that result in the generation of multiple phenotypes with different drug-metabolizing capabilities. Genetically derived differences can easily be assessed by genotyping assays in cases where the polymorphism has been identified. However, many of the polymorphisms that result in these are not known, secondly not all the differences can be attributed to genetic polymorphisms, hence genotyping methods cannot be employed. We have therefore, developed real-time (Taqman) PCR assays to quantitate levels of P450 mRNAs in human tissues. These assays are highly sensitive, reproducible, and specific and will allow quantitation of P450 mRNA levels in various human tissues. We have applied these assays to quantitate cytochrome P450 mRNA levels in human skin samples from 27 healthy volunteers. The expression of 13 P450s was assessed. The major enzymes detected were CYP1B1, CYP2B6, CYP2D6, and CYP3A4 with mean values of 2.5, 2.6, 2.7, and 1.1 fg/18S rRNA in 50 ng total RNA, respectively. Lower levels of CYP2C18, CYP2C19, and CYP3A5 were also detected while CYP1A2, 2A6, and 2C8 were below limits of detection. There was interindividual variation in the levels of mRNA among the 27 subjects studied although Poisson analysis showed data to be normally distributed, except for CYP2B6, as some individuals completely lacked CYP2B6 mRNA.

Anesth. Analg. (1)


http://www.anesthesia-analgesia.org/cgi/content/abstract/98/6/1566

We sought to determine whether local administration of pentoxifylline (PTF) or propentofylline (PPTF), which hinders cytokine production, influences pain threshold and formalin-induced pain behavior in rats or the level of tumor necrosis factor-{alpha} (TNF-{alpha}) messenger RNA (mRNA) concentrations in the inflamed paw tissue. PTF (0.5, 1, or 2 mg) and PPTF (1 or 2 mg) injected intraplantarly (i.pl.) had no significant effect on pain threshold. Injection of 0.1 mL of a 12% formalin solution subcutaneously into the dorsal surface of the left hindpaw induced pain behavior (47.6 {+/-} 4.6 incidents per 5 min), and PTF injected at doses of 1 and 2 mg/100 {micro}L i.pl. before (but not after) formalin was effective in antagonizing (33.6 {+/-} 2.5 and 23.6 {+/-} 3.4 incidents per 5 min, respectively) formalin-induced pain behavior. A similar antagonistic effect was observed after PPTF treatment at a dose of 2 mg/100 {micro}L; however, in contrast to PTF, at a later time point (85-90 min) after the formalin challenge, this effect was independent of the scheme of PPTF administration, before or after formalin. The effect of PTF on formalin-induced pain behavior did not parallel paw volume as measured by plethysmometer; however, PTF per se significantly increased the paw volume. Formalin injection significantly increased the TNF-{alpha} mRNA level in the inflamed tissue of the rat hind paw (150%). PTF administered before, but not after, formalin significantly antagonized (by approximately 40%) the observed increase in the level of TNF-{alpha} mRNA. Our study demonstrates and provides biochemical evidence that preemptive inhibition of proinflammatory cytokine synthesis by the use of PTF and PPTF, phosphodiesterase, and glial activation inhibitors is useful in antagonizing hyperalgesia in formalin-induced pain. Moreover, local administration of PTF may be a valuable approach to the treatment of inflammatory pain. IMPLICATIONS: This study demonstrates and provides biochemical evidence that preemptive inhibition of proinflammatory cytokine synthesis by local
administration of pentoxifylline and propentofylline is useful in antagonizing hyperalgesia in formalin-induced pain. Moreover, local administration of pentoxifylline could be regarded as a valid approach to the treatment of inflammatory pain.

Animal Behaviour (1)


http://www.sciencedirect.com/science/article/B6W9W-4BMJVGF-3/2/92f2358ca837d6dcb64800bfd2dd8b2b

In natural populations of rodents, males are more likely to engage in aggression and be infected with hantaviruses than females. Whether the relationship between hantavirus infection and aggression is due to host- or parasite-mediated mechanisms is unknown. The aim of this study was to determine whether hantavirus infection causes an increase in aggression in male rats and whether these behavioural changes are due to infection of the central nervous system or peripheral tissues. Male laboratory rats were infected with Seoul virus and tested for aggression in a resident-intruder paradigm 15 and 30 days postinoculation (p.i.). Males tested 30 days p.i. (i.e. during the persistent phase of infection) spent more time engaged in aggression than either uninfected males or males tested during the acute phase of infection (i.e. 15 days p.i.). Males that engaged in aggression for a longer duration had more virus present in lung, kidney and testis than males that spent less time engaged in aggression. Infected males shed virus in saliva, faeces, and urine; virus shedding, however, was not correlated with aggression and neither wounding nor transmission of virus to intruder males occurred during behavioural tests. Infection with Seoul virus did not alter either testosterone or corticosterone concentrations. Seoul virus antigens were not detected in the brains of infected rats. These data suggest that hantavirus infection leads to elevated aggression in infected males and may be a by-product of increased virus replication in peripheral tissues.

Animal Reproduction Science (2)


http://www.sciencedirect.com/science/article/B6T43-4F3NXY2-1/2/c67b8a0651bb36a9ff4902d752efe068

Control of luteal regression in the dog is still poorly understood. Unlike other domestic animal species, luteolysis is not prevented by hysterectomy. Indications that PGF2[alpha] may act as an endogenous luteolytic agent have been found only in pregnant animals during the prepartal decline of progesterone. Evidence from several species indicates that the immune system plays an important role in corpus luteum (Cl) function, possibly by the release of cytokines from immigrant immune cells. Hence, in the present experiment we attempted to examine the
expression of cytokines in the canine Cl during the course of dioestrus (formation and regression of the Cl), using RT-PCR. Groups of 4-5 bitches were ovario-hysterectomised on days 5, 15, 25, 35, 45 and 60-80 after ovulation. Canine-specific primers for IL-1[beta], IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF[alpha], IFN[gamma] and TGF[beta]1 were used. Positive and negative controls were included in all tests. Adequate expression was confirmed by sequencing selective samples of positive PCR products. The expression of mRNA for IL-8, IL-10, IL-12, TNF-[alpha] and TGF-[beta]1 was detected in all samples at each stage of dioestrus, without any obvious variations indicating a likely modulatory function of these cytokines in differentiation, maintenance or regression of the canine Cl. All tests for the expression of mRNA for IL-4, IL-1[beta] and IL-2 were negative. More negative than positive results were obtained when testing for the expression of mRNA for IL-6 and IFN-[gamma], leading to the conclusion that expression of these two cytokines is at a low level, and no conclusion can be drawn as to their involvement in control of Cl function.


http://www.sciencedirect.com/science/article/B6T43-3S53VPS-5/2/3a75a6a9cf652ce34f06b0bc659d2357

The aim of this study was to carry out first trimester fetal sex diagnosis using the polymerase chain reaction (PCR) to amplify DNA from bovine fetal cells recovered by transvaginal ultrasound-guided uterine puncture and fetal fluid aspiration. For sex determination, a nested, allele-specific, PCR amplification of the bovine zfx and zfy gene fragments was utilised. The PCR assay was validated using fetal fluids recovered from uteri post mortem. Cells were harvested from the fetal fluids, genomic DNA extracted and the PCR assay applied. A technique which was developed for transvaginal ultrasound-guided follicle aspiration was modified to recover fetal fluid from live animals. Small volumes of fetal fluid (0.5-5 ml) were recovered between days 61-97 of gestation and the PCR assay applied. The gender determined by PCR of fetal fluid cells was in all cases confirmed by visual inspection (n = 15 abattoir specimens) or ultrasound scanning (n = 7 live animals). Fetal death, attributed to the introduction of intrauterine infection, occurred in 4/4 cows in the first series of aspirations but in only 1/3 heifers in the second series of aspirations.

Ann. Bot. (2)


http://aob.oupjournals.org/cgi/content/abstract/93/2/189

* Background and Aims Isolation and drift are the main causes for geographic structure of molecular variation. In contrast, the one found in a previous survey in Armeria (Plumbaginaceae) for nuclear ribosomal ITS multicopy regions was species-independent and has been hypothesized to be due to extensive gene-flow and biased concerted evolution. Since this was inferred from a genus-level phylogenetic analysis, the aim of this study was to check for the occurrence of such structure and the validity of the proposed model at a local scale, in a southern
Spanish massif (Sierra Nevada), as well as to examine the evolutionary implications at the organism level. * Methods In addition to 117 sequences of direct PCR products from genomic DNA, 50 sequences of PCR products from cloned DNA were obtained to analyse cases of intragenomic polymorphisms for the ITS regions. * Key Results Sequence data confirm the occurrence of a species-independent structure at a local scale and reveal insights through the analysis of contact areas between different ITS copies (ribotypes). A comparison between cloned and direct sequences (a) confirms that, within these contact areas, ITS copies co-occur both in different individuals and within single genomes; and (b) reveals recombination between different copies. * Conclusions This study supports the utility of direct sequences for detecting intra-individual polymorphism and for partially inferring the ITS copies involved, given previous knowledge of the variability. The main evolutionary implication at the organism level is that gene-flow and concerted evolution shape the geographic structure of ITS variation.


http://aob.oupjournals.org/cgi/content/abstract/89/1/83

In Arabidopsis thaliana expression of the B-class MADS-box genes APETALA3 (AP3) and PISTILLATA (PI) is confined to petals and stamens but in other plant species these genes are also transcribed in non-flower tissues; in Solanum tuberosum they are transcribed specifically in vascular bundles leading to petals and stamens. Transcription analysis of B-class genes in Eranthis hyemalis using reverse transcribed in situ PCR revealed that both AP3 and PI are expressed in developing vascular bundles in the tuberous rhizome, flowering stem and floral primordia. In addition, AP3 and PI transcripts are also found in stems and leaves. These results suggest a more complex role of B-class genes in Eranthis and possible involvement in the development of vascular tissue.


http://www.annclinlabsci.org/cgi/content/abstract/34/3/314

{alpha} (1,2)-Fucosyltransferase catalyzes the transfer of fucose to the C-2 position of galactose on type II precursor substrate Gal(beta)1-4GlcNAc(beta)1-R. It plays an important biological role in the formation of H antigen, a precursor oligosaccharide for both A and B antigens on red blood cells. Aberration of {alpha}(1,2)-fucosyltransferase activity by gene mutations results in decreased synthesis of H antigen, leading to the para-Bombay phenotype. In this study, we collected about 250,000 blood samples in Taiwan during 5 yr and identified the subjects with para-Bombay phenotype. Then we analyzed the sequence of the {alpha}(1,2)-fucosyltransferase gene by direct sequencing and gene cloning methods, using the blood samples of 30 para-Bombay individuals and 30 control subjects who were randomly selected. The goals of this study were to search for new h alleles, to determine the h allele frequencies, and to test whether the sporadic theory is applicable in Taiwan. Six different h alleles (ha, 547~548 AG-del; hb, 880~881 TT-del; hc,
R220C; hd, R220H; he, F174L; and hf, N327T) were observed. Two h alleles, he and hf, were newly discovered in Taiwan. The he allele has a nucleotide 522C>A point mutation, predicting the amino acid 174 substitution of Phe to Leu; the hf allele has missense mutation of nucleotide 980A>C, predicting the amino acid 327 substitution of Asn to Thr. Frequencies of the 6 alleles are ha 46.67%, hb 38.33%, hc 5.00%, hd 1.67%, he 3.33%, and hf 5.00%, respectively. These findings in the Taiwanese population confirm previous observations in other populations that the Bombay and para-Bombay phenotypes are due to diverse, sporadic, nonfunctional alleles, predominantly ha and hb, leading to H deficiency of red blood cells. In contrast to previous reports of non-prevalent associations of h alleles with para-Bombay phenotype, our results suggest a regional allele preference associated with para-Bombay individuals in Taiwan.

http://www.annclinlabsci.org/cgi/content/abstract/34/1/63

The ABO system is one of the major blood groups that have significant impact on blood transfusion and paternity testing. We have found a new ABO allele by analyses of the ABO genotype of the Taiwanese population. Exons 6 and 7 of the ABO gene were amplified by the polymerase chain reaction and analyzed by direct sequencing. The results indicated that the ABO gene in the Taiwanese population consists mainly of the A1, A1v, B, O1, and O1v alleles. In addition, a novel O allele designated as OTaiwan was identified that has G [-&gt;] T substitution at the nucleotide 801 of the O1 allele. The OTaiwan allele is inheritable, since it is also present in an offspring of the OTaiwan-carrying individual. The information presented herein is valuable for population research and for analyses of evolutionary lineage.

http://www.annclinlabsci.org/cgi/content/abstract/34/3/319

Trisomy 21 is the most common chromosomal aberration in live births. In this study we employed human chromosome 21-specific short tandem repeat (STR) DNA markers to determine the numbers of chromosome 21 present in fetal cells. Forty amniotic fluid samples from pregnancies complicated with fetal Down syndrome and 98 samples from euploid pregnancies were analyzed for D21S11 and interferon-{alpha} receptor (IFNAR) gene intervening sequence. Fluorescent dye-labeled primers were used in PCR amplification of these 2 markers. The PCR amplicon was analyzed with an automatic DNA sequence analyzer. The results showed that 35 of 40 fetal Down syndrome samples analyzed for IFNAR showed 3 distinct peaks, while 24 of 30 cases analyzed for D21S11 showed 3 distinct peaks. Two Down syndrome samples showed two uneven peaks. By analyzing 98 euploid pregnancies as controls, the ratios of area under the peaks were determined to be 1.31 {+/-} 0.22 and 1.96 {+/-} 0.18 (mean {+/-} SD) for the euploid pregnancies and pregnancies complicated by fetal Down syndrome with 2 peaks, respectively. Our data showed that altogether 39 of 40 (97.5%) Down syndrome cases were correctly identified based on either the 3-peak pattern in one or more of the DNA markers or the relative peak area ratio calculation. In conclusion, polymorphic STR DNA markers are useful for determining the numbers of chromosome 21 in fetal cells. The high sensitivity and automation of the procedures suggest a good prospect for use of this method in prenatal detection of fetal Down syndrome. However, this is a preliminary investigation and a large-scale study is necessary to validate the clinical application of this protocol.
Ann. N.Y. Acad. Sci. (13)


http://www.annalsnyas.org/cgi/content/abstract/1022/1/195

The human organism is continuously in close contact with microorganisms, especially bacteria. In the present work, by means of a real-time polymerase chain reaction (PCR) technique, we looked for the presence of a distinct bacterial gene in human cells. To this end, we cultured a human cell line, HL60, in a supernatant in which bacteria (Bacillus subtilis) had been grown. A transient transcession of bacterial DNA into the human cells was observed.


http://www.annalsnyas.org/cgi/content/abstract/967/1/258

Prior results from our genomic scan in Pima Indians indicated an obesity locus in a region on chromosome 11q23-24 that was also linked to diabetes. Bivariate linkage analysis for the combined phenotype "diabesity" gave the strongest evidence for linkage (LOD = 5.2). Our aim is to positionally clone the gene(s) responsible for the linkage. Linkage disequilibrium mapping is being used to narrow the chromosomal region. Single nucleotide polymorphisms (SNPs) are being systematically identified and genotyped at 50-kb intervals across the region of linkage. To date, 455 SNPs have been genotyped in 1229 Pimas. A region containing a cluster of SNPs strongly associated with BMI and a second region, approximately 2 Mb telomeric, containing a cluster of SNPs associated with diabetes have been preliminarily identified.


http://www.annalsnyas.org/cgi/content/abstract/1007/1/17

Estrogen plays an important role during midbrain development. This is indicated by the presence of nuclear estrogen receptors and the transient expression of the estrogen-forming enzyme aromatase. A number of recent studies have shown that estrogen promotes the differentiation and survival, as well as physiological performance, of midbrain dopaminergic cells. In addition, we have reported that both ways of cellular estrogen signaling (classical and nonclassical) as well as interactions with nonneuronal target cells are involved in the transmission of intra- and intercellular estrogen effects in this brain region. This study provides additional evidence that (i) estrogen is capable of regulating gene expression in cultured embryonic neurons and astrocytes differently and (ii) both signaling mechanisms, i.e., classically through nuclear receptors and nonclassically through the stimulation of membrane-estrogen receptors, which are coupled to distinct intracellular signal transduction cascades, contribute diversely to gene regulation. These
data reveal a high degree of complexity of estrogen action at the genomic level in the developing brain. Further studies are warranted to unravel the exact contribution of the differently regulated genes for developmental estrogen action.


http://www.annalsnyas.org/cgi/content/abstract/990/1/118

Vector-borne diseases are a potential public health threat to U.S. Forces Korea (USFK). Ehrlichia and Anaplasma spp., transmitted by ticks, are only two of several diseases that may affect military readiness and operations. Rodents were collected at selected U.S. military installations and training sites in the Republic of Korea. DNA was extracted from spleen tissues and assayed by PCR methods for Ehrlichia and Anaplasma species. From rodents and mustelids collected during 1999 and 2000, a total of 196 Apodemus agrarius (striped field mouse), 2 Mustela sibirica (weasel), and 1 Cricetulus triton nestor (Korean greater long-tailed hamster) were assayed for Ehrlichia and Anaplasma species-specific DNA fragments. Rodent surveillance indicated a very high prevalence of Ehrlichia and Anaplasma spp. at selected training sites. Ehrlichia/Anaplasma DNA were identified from spleen tissue from 157 Apodemus agrarius, 1 Mustela sibirica, and 1 Cricetulus triton nestor. Species-specific DNA fragments of E. canis (45), E. ewingii (16), A. phagocytophila (5), and A. platys (62) were amplified by PCR techniques. Seventy-one striped field mice had single infections, while 24 had mixed infections of 2 (17 specimens), 3 (7 specimens), or 4 (1 specimen) pathogens. The striped field mouse plays a role as a reservoir for latent infections of various Ehrlichia or Anaplasma species.


http://www.annalsnyas.org/cgi/content/abstract/1025/1/345

Analysis by differential display of genes induced in response to acute cocaine administration to rats revealed the significant downregulation of several mitochondrial genes in the cingulate cortex, including the subunits 1, 2, 4, 5, and 6 of NADH dehydrogenase and the subunit 2 of cytochrome c oxidase. Although the mechanism of the downregulation of expression of these mitochondrial genes by cocaine is presently not well understood, one can envisage that it involves an increased production of reactive oxygen species in cells of the cerebral cortex.


http://www.annalsnyas.org/cgi/content/abstract/1022/1/140

Bronchoscopy is a standard procedure in the workup of patients with suspicious pulmonary lesions. We wondered whether it is possible to isolate malignancy-associated mRNA from cell-free lavage supernatant. Extracellular mRNA from cell-free lavage supernatant of 25 patients with lung cancer (23 with non-small cell lung cancer, 2 with small cell lung cancer) was isolated, reverse-transcribed, and amplified by reverse transcriptase polymerase chain reaction. The quantity and quality of the isolated RNA were checked after cDNA synthesis by amplification with
{beta}-actin-specific primers. Afterwards, a panel of eight genes known to be expressed in lung tumors was used for the detection of tumor-associated mRNA expression in lavage supernatant and serum. mRNA coding for {beta}-actin could be isolated from lavage supernatant of all 25 patients. In addition, the expression of at least one tumor-associated gene was detectable in all patients. These results show that intact mRNA can be isolated from cell-free lavage supernatant and that its quantity and quality are sufficient for the detection of tumor-associated gene expression alterations. This may open new possibilities for the diagnosis of lung cancer.


http://www.annalsnyas.org/cgi/content/abstract/962/1/332

Immune stimulants, such as the bacterial endotoxin, lipopolysaccharide (LPS), the human immunodeficiency virus-1 coat protein gp120, or {beta}-amyloid peptides, lead to glial activation and production of various immune mediators, such as nitric oxide (NO) and proinflammatory cytokines in the brain. These mediators appear to contribute to neuronal cell death in neurodegenerative diseases. However, the signaling pathways, which mediate the neurotoxic effect by the endotoxin, are not understood. The purpose of this study was to determine the role of mitogen-activated protein kinase (MAPK) in LPS-induced neurodegeneration using mesencephalic dopaminergic neuron/glia cultures. We have found that the p38 MAPK is important in LPS-induced death of mesencephalic neurons in rat neuron-glia mixed cultures. Upon treatment with 10 ng/ml LPS, the number of dopaminergic neurons decreased by 80% within 48 h, preceded by a significant production of NO by glia. Neuroprotection by selective inhibition of p38 MAPK activity paralleled a decrease in LPS-induced inducible nitric oxide synthase (iNOS) expression. These events were significantly reduced by the selective p38 MAPK inhibitor, SB202190, but not by the inactive analogue SB202474. Inhibition of iNOS activity and NO production by treatment with GW274150 was also neuroprotective. Although the p38 MAPK inhibitor afforded significant neuroprotection from LPS toxicity in the neuron-glia mixed culture, it failed to protect dopaminergic neurons from 6-hydroxy-dopamine-induced toxicity, which acts directly on dopaminergic neurons by inducing hydroxyl radical formation from the mitochondria. The results suggest that p38 MAPK in glia plays a significant role in the LPS-induced death of mesencephalic neurons through induction of nitric oxide synthase and resulting NO production.


http://www.annalsnyas.org/cgi/content/abstract/990/1/302

Rickettsia prowazekii, the etiologic agent for epidemic typhus, and Borrelia recurrentis, the etiologic agent of relapsing fever, both utilize the same vector, the human body louse (Pediculus humanus), to transmit human disease. We have developed an assay to detect both bacterial pathogens in a single tube utilizing real-time PCR. Assays for both agents are specific. The R. prowazekii and B. recurrentis assays do not detect nucleic acid from R. typhi, R. canada, or any of eight spotted fever rickettsiae. In addition they did not react with Neorickettsia risticii, N. sennetsu, Franciscella persica, Bartonella quintana, Legionella pneumophila, Proteus mirabilis, Salmonella enterica, Escherichia coli, and Staphylococcus aureus. Moreover, the B. recurrentis assay did not detect B. duttonii, B. coriacae, B. afzelii, B. garinii, B. hermsii, or B. burgdorferi nucleic acid. Both assays detected repeatedly only R. prowazekii or B. recurrentis either when tested alone or together in one test tube.

http://www.annalsnyas.org


http://www.annalsnyas.org/cgi/content/abstract/1011/1/332

Long-term exposure to cadmium (Cd) induces perturbation of kidney proximal tubular epithelial cells. Mitochondrial dysfunction in renal cortical cells may contribute to the pathogenesis of Cd-induced nephropathy. In this study, we examined the accumulation of mitochondrial DNA (mtDNA) with a large deletion and cellular senescence in the renal cortex. Wistar rats at 8 weeks of age were intraperitoneally injected with 1 mL of 1 mM CdCl2 or saline, 3 times/week for 5, 20, 40, or 80 weeks. Mitochondrial Cd content in the renal cortex was quantified by atomic absorption analysis. Cytochrome c oxidase (CCO) and senescence-associated beta-galactosidase (SA-{beta}-gal) activity were determined in renal cortex by enzyme-histochemistry. mtDNA in total DNA extracted from the renal cortex was amplified by PCR, and mtDNA deletions, including 4,834-bp (nt8118-nt12937) deletion, were determined and semiquantified. After 40 weeks of Cd injection, Cd levels in the renal cortex reached a saturation level, and 30% of the level of the whole-cell fraction was found in the mitochondria. CCO activity in the renal cortex, which was predominantly found in proximal tubular cells, decreased after 40 weeks of Cd exposure. Expression of SA-{beta}-gal was detected primarily in the proximal tubular cells and significantly increased after 80 weeks of Cd exposure. After 40 weeks of study, accumulation of 4,834-bp deletion in mtDNA was evident in both groups of rats; however, the amount of the deletion was significantly greater in Cd-treated rats than in control rats. Our results indicate that long-term Cd exposure induced a post-regenerative state of proximal tubular cells, which accelerated accumulation of 4,834-bp mtDNA deletions in the renal cortex, suggesting that Cd may be a senescence acceleration factor for kidney proximal tubular epithelial cells, which results in Cd-induced nephropathy.


http://www.annalsnyas.org/cgi/content/abstract/967/1/71

We observed earlier that increased skeletal muscle lipid content in the hereditary hypertriglyceremic (hHTg) rat is accompanied by a decline in plasma leptin. Leptin has recently been shown to enhance peripheral insulin sensitivity by decreasing the tissue triglyceride accumulation, possibly through regulation of fatty acid oxidation and lipogenesis. Thus, to test the hypothesis that insulin resistance and increased skeletal muscle lipid accumulation in hHTg rats are due to a defect in lipid catabolism, we measured mitochondrial and peroxisomal fatty acid oxidation and malonyl-CoA and acetyl-CoA carboxylase-2 content in skeletal muscles of these animals. In addition, we investigated possible molecular mechanisms responsible for the lower leptin levels in hHTg rats by measuring leptin and leptin-receptor (Ob-Ra) mRNA levels. We found the following: (1) in spite of a higher skeletal muscle malonyl-CoA content and an increased sensitivity of carnitine palmitoyltransferase-1 to malonyl-CoA, carnitine palmitoyltransferase-1
activity in muscle of hHTg rats was normal; (2) increased peroxisomal fatty acidoxidation did not seem to be sufficient to prevent the tissue lipid accumulation in these animals; (3) both lower leptin production by white adipose tissue and increased leptin uptake seem to be responsible for lower circulating leptin levels and therefore lower fatty acid catabolism.


http://www.annalsnyas.org/cgi/content/abstract/967/1/558

Variability in the number of tandem repeats of the insulin gene (INS VNTR) is known to influence several phenotypes, including polycystic ovary syndrome (PCOS), diabetes mellitus type 1, diabetes mellitus type 2, and birth weight. The presence of the class III allele of INS VNTR has been reported to be protective in diabetes mellitus type 1, but in contrary it increases the disease risk of PCOS and diabetes mellitus type 2. PCOS is a very common endocrinopathy in women of reproductive age. The etiology of PCOS is uncertain, but family history of this syndrome suggests a major genetic cause. The aim of this pilot study was to investigate the possible association of INS VNTR polymorphism with PCOS in Czech women. In PCOS, significantly higher WHR, BMI, G0, G180, I30, Cp0, Cp30, Cp60, AUC-I, AUC-Cp, and insulinogenic index and significantly lower AUC-G/AUC-I were found. No significant differences in INS VNTR genotype, phenotype, or allele frequencies were found between PCOS and controls. In spite of several differences in anthropometric and biochemical parameters (abdominal fat localization, increased β-cell function, and lower insulin sensitivity in PCOS women), no effect of INS VNTR polymorphism was found on insulin secretion, insulin action, or any other screened parameter.


http://www.annalsnyas.org/cgi/content/abstract/1035/1/133

Serine racemase (SRace) is an enzyme that catalyzes the conversion of L-serine to pyruvate or D-serine, an endogenous agonist for NMDA receptors. Our previous studies showed that inflammatory stimuli such as Aβ could elevate steady-state mRNA levels for SRace, perhaps leading to inappropriate glutamatergic stimulation under conditions of inflammation. We report here that a proinflammatory stimulus (lipopolysaccharide) elevated the activity of the human SRace promoter, as indicated by expression of a luciferase reporter system transfected into a microglial cell line. This effect corresponded to an elevation of SRace protein levels in microglia, as well. By contrast, dexamethasone inhibited the SRace promoter activity and led to an apparent suppression of SRace steady-state mRNA levels. A potential binding site for NFκB was explored, but this sequence played no significant role in SRace promoter activation. Instead, large deletions and site-directed mutagenesis indicated that a DNA element between -1382 and -1373 (relative to the start of translation) was responsible for the activation of the promoter by lipopolysaccharide. This region fits the consensus for an activator protein-1 binding site. Lipopolysaccharide induced an activity capable of binding this DNA element in electrophoretic mobility shift assays. Supershifts with antibodies against c-Fos and JunB identified these as the responsible proteins. An inhibitor of Jun N-terminal kinase blocked SRace promoter activation, further implicating activator protein-1. These data indicate that proinflammatory stimuli utilize a signal transduction pathway culminating in activator protein-1 activation to induce expression of serine racemase.

http://annonc.oupjournals.org/cgi/content/abstract/13/10/1598

Background: Angiogenesis is essential for development, growth and advancement of solid tumors. ETS-1 has been recognized as a candidate for tumor angiogenic transcription factor. This prompted us to study the clinical implications of ETS-1-related angiogenesis in uterine cervical cancers. Patients and methods: Fifty patients underwent curative resection for uterine cervical cancers. The patients' prognoses were analyzed with a 24-month survival rate. In the tissue of 60 uterine cervical cancers, the levels of ets-1 mRNA, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF) and interleukin (IL)-8 were determined by competitive reverse transcription-polymerase chain reaction using recombinant RNA and enzyme immunoassay, and the localization and counts of microvessels were determined by immunohistochemistry. Results: There was a significant correlation between microvessel counts and ets-1 gene expression levels in uterine cervical cancers. Immunohistochemical staining revealed that the localization of ETS-1 was similar to that of vascular endothelial cells. The level of ets-1 mRNA correlated with the levels of PD-ECGF and IL-8 among angiogenic factors. Furthermore, the prognosis of the 25 patients with high ets-1 mRNA expression in uterine cervical cancers was extremely poor, while the 24-month survival rate of the other 25 patients with low ets-1 mRNA expression was 92%. Conclusions: ETS-1 might be a prognostic indicator as an angiogenic mediator in uterine cervical cancers.


http://annonc.oupjournals.org/cgi/content/abstract/15/3/489

Background: Hypermethylation is studied as a new, relevant mechanism for silencing tumor suppressor genes. It is a potentially reversible epigenetic change and it is the target of novel anticancer compounds with demethylating activity. In this perspective, we investigated E-cadherin gene (CDH1) promoter hypermethylation in gastric carcinomas and its correlation with E-cadherin protein expression. Methods: Consecutive cases of gastric carcinoma with assessable paraffin-embedded tumor blocks and paired normal mucosa were considered eligible for study entry. CDH1 promoter hypermethylation and E-cadherin protein expression were determined by methylation-specific polymerase chain reaction and immunohistochemistry, respectively. Results: CDH1 promoter hypermethylation was found in 20 out of 70 gastric carcinomas and the epigenetic change occurred in the early, as well as in the locally advanced disease. In five cases, hypermethylation was also detected in the normal mucosa. Eighteen out of 20 hypermethylated tumors were of the diffuse histotype (P = 0.0001). Of 24 tumors with reduced or negative E-cadherin expression, 19 were hypermethylated and 5 were unmethylated (P = 0.0001). Conclusions: CDH1 promoter hypermethylation frequently occurs in gastric carcinomas of the diffuse histotype and it is significantly associated with downregulated E-cadherin expression. The knowledge on the hypermethylation status of tumor suppressor genes may be relevant to the development of demethylating drugs and novel chemopreventive strategies in solid tumors.

http://annonc.oupjournals.org/cgi/content/abstract/14/5/704

Background: Mutations in the p53 gene are the most common genetic alterations in human primary breast carcinoma and these mutations are often associated with worse prognosis and chemo/radioresistance. Patients and methods: The analysis of the p53 gene was performed by fluorescence-assisted mismatch analysis in 13 consecutive high-risk primary breast cancer (HR-BC) patients with 10 or more involved axillary nodes to evaluate its prognostic value. Results: Three p53 mutations (23%) and four allelic variants were detected. After a median follow-up of 52 months the HR-BC disease-free survival (DFS) was 51% and overall survival 79%. All patients harboring a p53 mutation (p53mut) relapsed within 10 months of the median DFS while 67% of those showing a wild-type p53 status (p53wt) survive disease-free at a median follow-up of 43 months. One p53mut patient is still alive while all the p53wt patients survive at 56 months median follow-up. Two out of the four p53wt relapsing breast cancer patients showed the Arg72Pro allelic variant; one of these died at 75 months. Conclusions: p53 mutations may help identify a subset of very high risk breast cancer patients (vHR-BC) with worse prognosis.


http://annonc.oupjournals.org/cgi/content/abstract/15/1/151

Background: To evaluate the impact of dihydrofolate reductase (DHFR) and reduced folate carrier (RFC) genes on methotrexate (MTX) resistance in osteosarcoma cells in relation to retinoblastoma (RB1) gene status. Materials and methods: A series of human osteosarcoma cell lines—either sensitive or resistant to MTX—and 16 osteosarcoma tumour samples were used in this study. Results: In U-2OS MTX-resistant variants, and in other RB1-positive cell lines, MTX resistance was associated with increased levels of DHFR and with a slight decrease of RFC gene expression. In Saos-2 MTX-resistant variants, and in another RB1-negative cell line, development of MTX resistance was associated with a decrease in expression of RFC, without any significant involvement of DHFR. In osteosarcoma clinical samples, amplification of the DHFR gene at clinical onset appeared to be more frequent in RB1-positive compared with RB1-negative tumours. Conclusions: Amplification of the DHFR gene may occur more frequently in the presence of RB1-mediated negative regulation of its activity and can be present at clinical onset in osteosarcoma patients. Simultaneous evaluation of RFC, DHFR and RB1 gene status at the time of diagnosis may become the basis for the identification of potentially MTX-unresponsive osteosarcoma patients, who could benefit from treatment protocols with alternative antifolate drugs.


http://ard.bmjournals.com/cgi/content/abstract/64/5/780

Objective: To evaluate the role of parvovirus B19 (B19), varicella zoster virus (VZV), and human herpes virus 6 (HHV-6) in the aetiopathology of giant cell arteritis (GCA). Methods: Temporal artery biopsy specimens from 57 patients with GCA and 56 controls were investigated. DNA was obtained by biopsy, and quantitative real time polymerase chain reaction assay performed to establish the prevalence and viral load of B19, VZV, and HHV-6. Amplification of the human \{beta\}-globin gene was used as internal positive control. Results: (a) B19 was detected in 31/57 (54%) patients (median viral load 45.2 (25th-75th centiles 0-180.2) copies/{micro}g DNA) v 21/56 (38%) controls (median viral load 0 (0-66.7) copies/{micro}g of DNA; p = 0.07 for DNA prevalence, p = 0.007 for viral load. Among 31 B19 positive samples, 21 (68%) patients with biopsy proven GCA had >102 B19 copies/{micro}g of DNA v 5/21 (24%) controls; p = 0.001. (b) No significant difference was found for VZV (p = 0.94 for DNA prevalence; p = 0.76 for viral load) and HHV-6 (p = 0.89 for DNA prevalence; p = 0.64 for viral load) in the GCA group compared with controls. Conclusion: B19 may have a role in the aetiopathology of GCA, particularly in those patients with high viral load; no evidence was found for VZV and HHV-6.


http://ard.bmjournals.com/cgi/content/abstract/62/10/983

Background: Transgenic deficiency in interferon \{gamma\} (IFN{gamma}) or IFN{gamma} receptor makes resistant strains of mice bearing H-2b or H-2d susceptible to collagen induced arthritis (CIA). Objective: To determine whether the escape from regulation of disease susceptibility at the major histocompatibility complex level involves a new use of autoimmune T cells expressing T cell receptor (TCR) V{beta} that vary from the cell populations previously identified within arthritic joints. Methods: Arthritis was induced by a standard protocol with type II bovine collagen (CII) in complete Freund’s adjuvant. Clinical features, histopathology, immunological responses, and TCR profile in arthritic joints in IFN{gamma} knockout C57BL/6 (B6.IFN{gamma} KO) mice (H-2b) were compared directly with those in DBA/1 mice (H-2q). Results: 60-80% of B6.IFN{gamma} KO mice developed a progressive arthritis with a similar clinical course to classical CIA in DBA/1 mice. The affected joints in B6.IFN{gamma} KO mice had an erosive form of arthritis with similar features to joint disease in DBA/1 mice. B6.IFN{gamma} KO mice produced significantly higher levels of IgG2b and IgG1 autoantibodies to murine CII and showed increased proliferative response to CII compared with B6 mice. Comparable levels of interleukin 1{beta} and tumour necrosis factor \{alpha\} expression were detected in arthritic joints from \{beta\}6.IFN{gamma} KO and DBA/1 mice. B6.IFN{gamma} KO mice used predominantly TCR V{beta}6 and V{beta}8 in arthritic joints. This TCR V{beta} profile is similar to that found in DBA/1 mice with CIA. Conclusions: C57BL/6 mice deficient in IFN{gamma} production can develop arthritis that resembles classical CIA. These data suggest that IFN{gamma} is a key factor mediating susceptibility to CIA.

Background: Antiphospholipid antibodies reacting with \(\beta_2\)-glycoprotein I (\(\beta_2\)GPI) have been associated with recurrent fetal loss and pregnancy complications. Objective: To investigate whether specific mutations in the phospholipid binding site of \(\beta_2\)GPI might affect its binding to trophoblast and in turn the anti-\(\beta_2\)GPI antibody induced functional effects. Methods: \(\beta_2\)GPI adhesion to trophoblast was evaluated as human monoclonal IgM or polyclonal IgG anti-\(\beta_2\)GPI antibody binding to trophoblast monolayers cultured (1) in complete medium; (2) in serum-free medium; (3) after serum starvation in the presence of purified human \(\beta_2\)GPI; or (4) in the presence of \(\beta_2\)GPI with single or multiple mutations in the amino acid loop Cys281-Lys-Asn-Lys-Glu-Lys-Lys-Cys288. The effect of anti-\(\beta_2\)GPI binding to trophoblast was evaluated as chorionic gonadotropin (hCG) mRNA expression, and protein release by RT-PCR and radioimmunoassay, respectively. Results: \(\beta_2\)GPI adhesion to trophoblast and its consequent recognition by the specific antibodies were inversely proportional to the mutation number in the phospholipid binding site. Anti-\(\beta_2\)GPI antibodies reduced gonadotropin release, hormone dependent hCG mRNA expression, and protein synthesis in the presence of \(\beta_2\)GPI, while the addition of the mutants or the absence of \(\beta_2\)GPI had no effect. Conclusions: \(\beta_2\)GPI binds to trophoblast in vitro through its fifth domain, as reported for endothelial cells, and can be recognised by anti-\(\beta_2\)GPI antibodies; the antibody binding downregulates trophoblast hCG synthesis and secretion. Such a mechanism might contribute to defective placentation in women with fetal loss associated with the antiphospholipid syndrome.


http://ard.bmjjournals.com/cgi/content/abstract/64/2/279

Background: Chronic recurrent multifocal osteomyelitis (CRMO) in children is a chronic non-suppurative inflammation involving multiple sites. Some children affected by chronic non-bacterial osteomyelitis (CNO) do not have multiple lesions or a recurrent course. Objective: To characterise the long term outcome of children with the full spectrum of CNO. Methods: 30 children diagnosed with CNO were followed up for a mean of 5.6 years and their disease assessed using a clinical score, multiple imaging, and a diagnostic biopsy, including extensive microbial analysis. Results: 9 patients had unifocal non-relapsing disease, 3 unifocal lesions with relapses, 9 multifocal lesions without relapses, and 9 multifocal lesions with relapses (CRMO). Granulocytes were present significantly more often in CRMO than in unifocal and non-recurrent lesions. Pustulosis was more common in multifocal cases regardless of recurrence. Mean duration of treatment in 15 children with a single occurrence was 9.2 months. Naproxen treatment was generally effective. Naproxen treatment in 12 patients with relapses lasted 25 months. However, 7 of these were not effectively treated with naproxen alone. Five were treated with oral glucocorticoids for 27 days in addition to naproxen, which induced remission in four, lasting for at least 1.5 years. Longitudinal growth of affected bones was not altered, except for the development of hyperostosis. Conclusion: CNO is a spectrum of inflammatory conditions, with CRMO being the most severe. Most children with CNO have a favourable outcome of the disease. Oral glucocorticoids may be necessary in severe recurrent cases.


http://ard.bmjjournals.com/cgi/content/abstract/64/3/477
Background: Expression of aggrecan is reduced during aging and osteoarthritic cartilage degeneration. CpG methylation may have a role in the down regulation of aggrecan transcriptions. Objective: To investigate whether a correlation between gene methylation and expression of aggrecan in chondrocytes exists. Methods: The human aggrecan promoter region was analysed computationally for CpG-rich regions. These were investigated for the methylation of C residues in normal (aged) and osteoarthritic chondrocytes by the bisulphite method for modifying DNA as well as sequence analysis using DNA directly extracted from normal and osteoarthritic cartilage tissue. Additionally, chondrocytic cell lines were investigated for methylation within the aggrecan promoter region. Results: The CpG-rich promoter region of the human aggrecan gene contains a 0.6 kb region that meets the criteria of a CpG island as defined by prediction programmes. A significant correlation of aggrecan mRNA expression levels and methylation status in normal (aged) and osteoarthritic chondrocytes as well as in different chondrocytic cell lines was not found. Conclusions: Expression of aggrecan in normal cartilage and diseased states is not modulated by gross changes of CpG methylation of its promoter region. CpG methylation does not have a central role in the switch off of aggrecan promoter activity in human adult articular chondrocytes.


http://ard.bmjournals.com/cgi/content/abstract/61/7/591

Objective: To characterise the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) during degeneration of articular cartilage in a transgenic Del1 mouse model for osteoarthritis. Methods: Northern analysis was used to measure mRNA levels of MMP-2, -3, -8, -9, -13, and -14, and TIMP-1, -2, and -3 in total RNA extracted from knee joints of transgenic Del1 mice, harbouring a 15 amino acid deletion in the triple helical domain of the {alpha}1(II) collagen chain, using their non-transgenic littermates as controls. Immunohistochemistry was used to study the presence of cleavage products (neoepitopes) of type II collagen, and the distribution of MMP-13 and TIMP-1 in degenerating cartilage. Results: Each of the MMP and TIMP mRNAs analysed exhibited distinct expression patterns during development and osteoarthritic degeneration of the knee joint. The most striking change was up regulation of MMP-13 mRNA expression in the knee joints of Del1 mice at the onset of cartilage degeneration. However, the strongest immunostaining for MMP-13 and its inhibitor TIMP-1 was not seen in the degenerating articular cartilage but in synovial tissue, deep calcified cartilage, and subchondral bone. The localisation of type II collagen neoepitopes in chondrocytes and their pericellular matrix followed a similar pattern; they were not seen in cartilage fibrillations, but in adjacent unaffected cartilage. Conclusion: The primary localisation of MMP-13 and TIMP-1 in hyperplastic synovial tissue, subchondral bone, and calcified cartilage suggests that up regulation of MMP-13 expression during early degeneration of articular cartilage is a secondary response to cartilage erosion. This interpretation is supported by the distribution of type II collagen neoepitopes. Synovial production of MMP-13 may be related to removal of tissue debris released from articular cartilage. In the deep calcified cartilage and adjacent subchondral bone, MMP-13 probably participates in tissue remodelling.


http://ard.bmjournals.com/cgi/content/abstract/64/4/575
Objectives: To analyse the association of interleukin 10 (IL10) promoter polymorphisms, which have been shown to be related to IL10 secretion capacity, with the response to long term treatment with etanercept in patients with rheumatoid arthritis (RA). Methods: Fifty patients with active RA were treated for up to 4 years (median 39 months, range 3-52) with stable doses of etanercept as monotherapy. Treatment response was assessed as defined by the EULAR criteria in an intention to treat analysis, with the last observation carried forward. IL10 promoter microsatellite polymorphisms IL10.R and IL10.G were genotyped by fragment length analysis in patients and 189 healthy controls matched for ethnicity, age, and sex. Haplotypes were reconstructed using a method based on bayesian, coalescent theory with the PHASE software. Results: IL10 microsatellite polymorphisms were not associated with susceptibility to RA. When patients with good treatment response (n = 25) were compared with patients with moderate (n = 17) or no response (n = 8), a significantly different distribution of the prevailing alleles R2, R3 and G9, G13, respectively, became evident. Good treatment response was associated with carriage of the R3 allele or R3-G9 haplotype, whereas the allele G13 and the haplotype R2-G13 predominated in patients with moderate or no response. Conclusion: Genotyping of the IL10 promoter microsatellites may be useful in predicting the clinical response to etanercept in patients with RA. The high prevalence of the presumptive IL10 low producer allele R3 in patients with a favourable response suggests that IL10 promotes disease activity in RA under the specific condition of tumour necrosis factor antagonism.


http://ard.bmjournals.com/cgi/content/abstract/61/8/741

Objective: To gain a better understanding of how iron accumulates in human rheumatoid synovium. Methods: The distribution of ferritin, transferrin receptor, and non-specific resistance associated macrophage proteins 1 and 2 (Nramp1 and Nramp2) in the human rheumatoid synovium was investigated by immunocytochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR). Results: Both heavy and light ferritin subunit types were detected in the lining layer and the subintimal zone of rheumatoid synovium, heavy ferritin generally being more abundant than light. Both heavy and light ferritin were detected in isolated synovial macrophages and fibroblasts. Transferrin receptor expression was largely confined to fibroblasts of the synovial lining layer. Nramp2 was detected by PCR in both isolated synovial macrophages and fibroblasts, whereas Nramp1 was detected by PCR and immunocytochemistry in macrophages and neutrophils in the lining and subintimal zone, and in inflammatory infiltrates, but was absent from fibroblasts. Conclusion: A complex chain of events, perhaps initiated by proinflammatory cytokines, may culminate in a toxic build up of iron in the rheumatoid joint.

Ann. Surg. Oncol. (7)


http://www.annalsurgicaloncology.org/cgi/content/abstract/11/9/861
Background: Theory holds that the upper outer quadrant of the breast develops more malignancies because of increased tissue volume. This study evaluated genomic patterns of loss of heterozygosity (LOH) and allelic imbalance (AI) in non-neoplastic tissues from quadrants of diseased breasts following mastectomy to characterize relationships between genomic instability and the propensity for tumor development. Methods: Tissues from breast quadrants were collected from 21 patients with various stages of breast carcinoma. DNA was isolated from non-neoplastic tissues using standard methods and 26 chromosomal regions commonly deleted in breast cancer were examined to assess genomic instability. Results: Genomic instability was observed in breast quadrants from patients with ductal carcinomas in situ and advanced carcinomas. Levels of instability by quadrant were not predictive of primary tumor location (P = .363), but outer quadrants demonstrated significantly higher levels of genomic instability than did inner quadrants (P = .017). Marker D8S511 on chromosome 8p22-21.3, one of the most frequently altered chromosomal regions in breast cancer, showed a significantly higher level of instability (P = .039) in outer compared with inner quadrants. Conclusions: Non-neoplastic breast tissues often harbor genetic changes that can be important to understanding the local breast environment within which cancer develops. Greater genomic instability in outer quadrants can partially explain the propensity for breast cancers to develop there, rather than simple volume-related concepts. Patterns of field cancerization in the breast appear to be complex and are not a simple function of distance from a developing tumor.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/3/297

Background: Heparan sulfate proteoglycans, the main components of the extracellular matrix, are recognized as important components of signal transduction and play an important role in tumor progression. Heparanase (hep) degrades heparan sulfate proteoglycans, but the clinical importance of hep is unclear. In this study, we investigated the clinicopathologic importance of hep messenger RNA (mRNA) expression in esophageal squamous cell carcinoma (ESCC). Methods: Fresh tumors and noncancerous epithelia were obtained from 57 ESCC patients after esophagectomy. Expression levels of hep and glyceraldehyde-3-phosphate dehydrogenase mRNA were quantitatively analyzed by real-time reverse transcriptase-polymerase chain reaction. Apoptotic cancer cells and microvessel density were evaluated immunohistochemically. Results: The relative hep mRNA expression level (hep:glyceraldehyde-3-phosphate dehydrogenase ratio) in ESCC was lower than in noncancerous tissue (P < .001). Tumor hep expression decreased according to tumor progression and correlated with the occurrence of apoptotic cancer cells, but not with tumor microvessel density. Moreover, low hep expression correlated with poor patient survival. Conclusions: Reduced hep mRNA expression might result in abnormal cell growth and correlate with ESCC progression.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/9/1086

Background: This study evaluated the relationship between DNA aneuploidy and loss of heterozygosity (LOH) at different genetic loci in colorectal adenocarcinoma. Methods: A total of 112 patients with surgically removed colorectal adenocarcinoma in Taipei Veterans General Hospital from January 1999 to July 2001 were included in this study. The pattern of DNA ploidy was determined with DNA flow cytometry, and the LOH of various genetic loci was determined with fluorescence polymerase chain reaction and denaturing gradient gel electrophoresis. The
relationship between DNA ploidy, LOH of various genetic loci, and clinicopathologic variables was analyzed with the χ² test with Yates' correction as well as by multivariate binary logistic regression analysis. Results: Seventy-one (63.4%) of the 112 carcinomas had DNA aneuploidy. The DNA aneuploidy was not associated with any clinicopathologic variable. Ninety-one tumors (81.3%) exhibited LOH in at least one genetic locus. In the univariate analysis, the DNA aneuploidy was associated with LOH of Tp53-penta, D8S254, D5S346, and high-frequency LOH (P = .001, P = .016, P = .041, and P < .001, respectively). In the multivariate analysis, the most significant factor influencing DNA aneuploidy was D8S254, followed by Tp53-penta, high-frequency LOH, and D5S346. Conclusions: DNA aneuploidy is strongly associated with LOH at specific genetic loci.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/2/136

Background: The aim of this study was to more precisely map the region of 16q loss of heterozygosity (LOH) in Wilms' tumors and to examine the expression of putative tumor suppressor. Methods: We performed polymerase chain reaction-based LOH analysis on the 185 sample pairs from 21 to 80 megabases (Mb) on chromosome 16q. Expression of two candidate tumor suppressor genes located within the identified consensus region of 16q LOH was examined by immunohistochemistry. Results: We identified 16q LOH in 7 (4%) of 185 Wilms' tumors not previously thought to demonstrate such genetic loss. The smallest common region of genetic loss was located between 67.3 and 74.0 Mb on chromosome 16. Within this 6.7-Mb region, there reside only three recognized tumor suppressor genes: E-cadherin, P-cadherin, and E2F4. E-cadherin demonstrates statistically significantly reduced expression in Wilms' tumors with 16q LOH. Conclusions: We have localized the consensus region of 16q LOH in Wilms' tumor to a 6.7-Mb locus and have identified three candidate Wilms' tumor suppressor genes within this narrowed region. Our data support E-cadherin as a candidate tumor suppressor gene in Wilms' tumor; however, further studies are needed to definitively prove its role as the tumor suppressor gene associated with 16q LOH.


http://www.annalssurgicaloncology.org/cgi/content/abstract/9/1/71

Background: We investigated tumor DNA changes before and after mastectomy in the plasma of breast cancer patients with no disseminated disease and eventually investigated these changes' relationship to specific pathological parameters of the tumors. Methods: We studied 41 patients. DNA extracted from tumor and normal breast tissues, mononuclear blood cells, and plasma was used for molecular studies. Alterations in the microsatellite markers D17S855, D17S654, D16S421, TH2, D10S197, and D9S161, as well as point mutations in the p53 gene and aberrant methylation of p16INK4a, were used to identify and characterize tumor and plasma DNA. A number of tumor clinicopathological parameters were analyzed in each patient. Results: We found that 18 (44%) of the 27 patients with alterations in tumor DNA presented the same plasma DNA alteration before mastectomy, and persistence of the same molecular features was detected in plasma DNA 4 to 6 weeks postmastectomy in 8 (19.5%) patients. Patients with vascular invasion, more than three lymph node metastases, and higher histological grade at diagnosis displayed plasma DNA after mastectomy with a significant difference. Conclusions: Persistence of plasma DNA with features of tumor DNA may be present after mastectomy in breast cancer.
patients, and its relation to bad-prognosis histological parameters may suggest undetectable micrometastatic disease.


http://www.annalssurgicaloncology.org/cgi/content/abstract/9/1/88

Background: T-cell receptor {gamma} (TCR-{gamma}) is involved in maintaining host cell integrity and homeostasis of the human immune system. We hypothesize that polymorphism of the TCR-{gamma} complex may be involved in the pathogenesis of colorectal cancer. Methods: The microsatellite markers D7S1818 and D7S2206 located within the TCR-{gamma} antigen locus on chromosome 7p were amplified by polymerase chain reaction, and genotypes were determined for 22 patients with early onset of colorectal cancer (<60 years old) and for 38 population-based control subjects. Results: Genotype BC of D7S1818 (P =.049) and haplotype AC of D7S1818/D7S2206 (P [.003) were associated with colorectal cancer as compared with the control population (extended Fisher's exact test). Conclusions: This study identifies a novel genetic and clinical association between TCR-{gamma} and early-onset colorectal cancer. Many young patients do not fulfill the criteria for hereditary colorectal cancer syndromes and are therefore not identified by established screening programs. Markers such as D7S1818 and D7S2206 may become useful in the identification of patients at risk of developing colorectal cancer and permit earlier therapeutic intervention.


http://www.annalssurgicaloncology.org/cgi/content/abstract/11/10/934

Background: Cancer-testis antigens (CTA), such as MAGE, are selectively expressed in various types of human neoplasms but not in normal tissues other than testis. This characteristic feature of CTA makes them promising antigens for cancer-specific immunotherapy. Methods: We investigated the expression of five genes, including MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4, in 20 surgical samples of intrahepatic cholangiocarcinomas (IHCC) using reverse transcription-polymerase chain reaction. To visualize the localization of MAGE proteins, we performed immunohistochemical studies. Furthermore, the correlation between the CTA expression and DNA methylation status was studied in three bile duct cancer cell lines. Results: Expression of MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4 was recognized in 4, 4, 2, 6, and 3 of all 20 cases, respectively. In contrast, the expressions of five genes were not recognized at all in the corresponding normal tissues. In 10 cases (50%), the tumors expressed at least one of the five CTA. An immunohistochemical analysis of MAGE proteins demonstrated homogenous or focal distributions in cytoplasm of the IHCC. Using a demethylating agent, MAGE-1, NY-ESO-1, SCP-1, and SSX-4 were induced in two of three cell lines, whereas MAGE-3 was not. Conclusions: Half of the tumor tissues of IHCC expressed at least one of the CTA. Some of the patients with IHCC, therefore, should be candidates for potentially useful cancer-specific immunotherapy.
Holt-Oram syndrome (HOS) is a specific developmental defect involving upper limb malformations and cardiac defects. Mutations in the TBX5 gene, located on chromosome 12q24.1, were demonstrated as the underlying molecular defect in several families with this disorder. We report on two unrelated families with HOS. Affected members of both families have the same truncation mutation in exon 5 of the TBX5 gene (Y136X). This mutation has not been reported before in HOS. The spectrum of defects is similar in both families, displaying an ASD, hypoplastic deltoid muscles and hypoplastic or absent thumbs extending to radial defects in one case. So far, only a single genotype-phenotype analysis in HOS has been done which is not sufficient to explain the high inter- and intrafamilial variability of expression. Our observation further supports that the position of the mutation in the TBX5 gene is related to the phenotype expression of HOS.


PurposeTo investigate whether the three single nucleotide polymorphisms (SNPs), SNP-43, -56, and -63 of CAPN10 were associated with type 2 diabetes in a West African cohort.MethodsA total of 347 diabetic subjects and 148 unaffected controls from four ethnic groups in two West African countries were enrolled in this study. After genotyping three SNPs of CAPN10 and one SNP from CYP19, the allele, genotype, and haplotype frequencies as well as the odds ratios were calculated to test their association with type 2 diabetes.ResultsNone of the alleles or genotypes was associated with type 2 diabetes. Although statistical analysis indicated that haplotype 221 was associated with type 2 diabetes (OR, 3.765; 95% CI, 1.577-8.989) in the two ethnic groups of Nigeria, the same haplotype did not show any association with type 2 diabetes in the two ethnic groups in Ghana (OR, 0.906; 95% CI, 0.322-2.552).ConclusionConsidering the relatively low frequency of haplotype 221 and that none of the haplotypes including 221 was associated with any of the diabetes-related quantitative traits tested, it is concluded that SNP-43, -56, and -63 of the CAPN10 gene variants may play a limited role in the risk of type 2 diabetes risks in this cohort of West Africans.
Purpose
Genetically determined mixture information can be used as a surrogate for physical or behavioral characteristics in epidemiological studies examining research questions related to socially stigmatized behaviors and horizontally transmitted infections. A new measure, the probability of mixture discrimination (PMD), was developed to aid mixture analysis that estimates the ability to differentiate single from multiple genomes in biological mixtures.

Methods
Four autosomal short tandem repeats (STRs) were identified, genotyped and evaluated in African American, European American, Hispanic, and Chinese individuals to estimate PMD. Theoretical PMD frameworks were also developed for autosomal and sex-linked (X and Y) STR markers in potential male/male, male/female and female/female mixtures.

Results
Autosomal STRs genetically determine the presence of multiple genomes in mixture samples of unknown genders with more power than the apparently simpler X and Y chromosome STRs. Evaluation of four autosomal STR loci enables the detection of mixtures of DNA from multiple sources with above 99% probability in all four racial/ethnic populations.

Conclusions
The genetic-based approach has applications in epidemiology that provide viable alternatives to survey-based study designs. The analysis of genes as biomarkers can be used as a gold standard for validating measurements from self-reported behaviors that tend to be sensitive or socially stigmatizing, such as those involving sex and drugs.

Antimicrob. Agents Chemother. (40)


http://aac.asm.org/cgi/content/abstract/47/11/3506

In 1999, 39 of 2,599 isolates of the family Enterobacteriaceae (1.5%) collected by eight private laboratories in the Aquitaine region in France produced an extended-spectrum \{beta\}-lactamase (ESBL). Among these were 19 Enterobacter aerogenes isolates; 8 Klebsiella pneumoniae isolates; 6 Escherichia coli isolates; 3 Proteus mirabilis isolates; and 1 isolate each of Serratia marcescens, Morganella morganii, and Providencia stuartii. ESBL producers were isolated from 38 patients, including 33 residents of 11 clinics or nursing homes and 5 ambulatory patients. Seven different ESBLs were characterized. These mainly consisted of TEM-24 (25 isolates) and TEM-21 (9 isolates), but TEM-15 (2 isolates) and TEM-3, TEM-19, SHV-4, and CTX-M-1 (1 isolate each) were also characterized. Seven strains showed the coexistence of different TEM- and/or SHV-encoding genes, including a new SHV-1 variant, SHV-44, defined by the substitution R205L previously reported for SHV-3 in association with S238G. The epidemiology of the ESBL producers was investigated by random amplification of polymorphic DNA, typing by enterobacterial repetitive intergenic consensus PCR, analysis of resistance cotransferred with the ESBL, and analysis of the restriction profiles of the ESBL-encoding plasmids. Of the TEM-24-expressing strains, 18 were E. aerogenes isolates, including 9 from the same clinic, that were representatives of the epidemic clone disseminating in France. Of the TEM-21-producing strains that belonged to different species of the family Enterobacteriaceae (E. coli, K. pneumoniae, and P. mirabilis), 8 were isolated in the same nursing home. Outbreaks due to strain and/or plasmid dissemination in these clinic and nursing home were demonstrated. The presence of ESBL producers in five ambulatory patients probably resulted from nosocomial acquisition. Our data highlight the serious need to monitor patients for ESBL-producing Enterobacteriaceae in general practice.
Enterobacter cloacae Ecl261 was isolated with Escherichia coli Ec257 from the urine of a patient living in a nursing home. Both isolates were resistant to ticarcillin (MICs, 1,024 (micro)g/ml), without significant potentiation of its activity by 2 (micro)g of clavulanate per ml (MICs, 512 (micro)g/ml), and susceptible to naturally active cephalosporins. This inhibitor-resistant phenotype was conferred in both strains by similar conjugal plasmids of 40 kb (Ecl261) and 30 kb (Ec257), which also conveyed resistance to sulfonamides and trimethoprim. Clinical and transconjugant strains produced a (beta)-lactamase with a pl of 5.2 which belonged to the TEM family, as indicated by specific PCR amplification. Compared with TEM-1, this enzyme exhibited lower catalytic efficiencies (14- and 120-fold less for amoxicillin and ticarcillin, respectively), and higher concentrations of (beta)-lactamase inhibitors were required to yield a 50% reduction in benzylpenicillin hydrolysis (750-, 82-, and 50-fold higher concentrations for clavulanate, sulbactam, and tazobactam, respectively). Gene sequencing revealed four nucleotide differences with the nucleotide sequence of blaTEM-1A. The first replacement (T32C), located in the promoter region, was described as being responsible for the increase in the level of (beta)-lactamase production. The other three changes led to amino acid substitutions that define a new inhibitor-resistant TEM (IRT) (beta)-lactamase, TEM-80 (alternate name, IRT-24). Two of them, Met69Leu and Asn276Asp, have previously been related to inhibitor resistance. The additional mutation, Ile127Val, was demonstrated by site-directed mutagenesis to have a very weak effect, at least alone, on the IRT phenotype. This is the first description of an IRT (beta)-lactamase in E. cloacae. The horizontal transfer of blaTEM-80 may have occurred either from Ec257 to Ecl261 or in the reverse order.


We assessed the sensitivities and specificities of three previously described PCR primers on enrichment broth cultures of feces for the accurate detection of fecal carriage of vancomycin-resistant enterococci (VRE). In addition, we investigated specimens that were vanB PCR positive but VRE culture negative for the presence of other vanB-containing pathogens. Feces from 59 patients (12 patients carrying vanB Enterococcus faecium strains and 47 patients negative for VRE carriage) were cultured for 36 h in aerobic brain heart infusion (BHI) broth, anaerobic BHI (AnO2BHI) broth, or aerobic Enterococcosel (EC) broth. DNA was extracted from the cultures and tested for the presence of vanB by using the PCR primers of Dutka-Malen et al. (S. Dutka-Malen, S. Evers, and P. Courvalin, J. Clin. Microbiol. 33:24-27, 1995), Bell et al. (J. M. Bell, J. C. Paton, and J. Turnidge, J. Clin. Microbiol. 36:2187-2190, 1998), and Stinear et al. (T. P. Stinear, D. C. Olden, P. D. R. Johnson, J. K. Davies, and M. L. Grayson, Lancet 357:855-856, 2001). The sensitivity (specificity) of PCR compared with the results of culture on BHI, AnO2BHI, and EC broths were 67% (96%), 50% (94%), and 17% (100%), respectively, with the primers of Dutka-Malen et al.; 92% (60%), 92% (45%), and 92% (83%), respectively, with the primers of Bell et al.; and 92% (49%), 92% (43%), and 100% (51%) respectively, with the primers of Stinear et al. The primers of both Bell et al. and Stinear et al. were significantly more sensitive than those of Dutka-Malen et al. in EC broth (P = 0.001 and P < 0.001, respectively). The poor specificities for all primer pairs were due in part to the isolation and identification of six anaerobic gram-positive...
bacilli, Clostridium hathewayi (n = 3), a Clostridium innocuum-like organism (n = 1), Clostridium bolteae (n = 1), and Ruminococcus lactaris-like (n = 1), from five fecal specimens that were vanB positive but VRE culture negative. All six organisms were demonstrated to contain a vanB gene identical to that of VRE. VanB-containing bowel anaerobes may result in false-positive interpretation of PCR-positive fecal enrichment cultures as VRE, regardless of the primers and protocols used.


http://aac.asm.org/cgi/content/abstract/46/3/680

The in vitro activities of ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin against a large collection of clinical isolates of Streptococcus pneumoniae (n = 4,650) obtained over a 5-year period, 1994-1995 through 1999-2000, were assessed as part of a longitudinal multicenter U.S. surveillance study of antimicrobial resistance. Three sampling periods were used during this investigation, the winter seasons of 1994-1995, 1997-1998, and 1999-2000; and 1,523, 1,596 and 1,531 isolates were collected during these three periods, respectively. The overall rank order of activity of the four fluoroquinolones examined in this study was moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin, in which moxifloxacin (MIC at which 90% of isolates are inhibited [MIC90], 0.25 {micro}g/ml; modal MIC, 0.12 {micro}g/ml) was twofold more active than gatifloxacin (MIC90, 0.5 {micro}g/ml; modal MIC, 0.25 {micro}g/ml), which in turn was fourfold more active than either levofloxacin (MIC90, 1 {micro}g/ml; modal MIC, 1 {micro}g/ml) or ciprofloxacin (MIC90, 2 {micro}g/ml; modal MIC, 1 {micro}g/ml). Changes in the in vitro activities of fluoroquinolones against S. pneumoniae strains in the United States over the 5-year period of the survey were assessed by comparing the MIC frequency distributions of the study drugs against the isolates obtained during the three sampling periods encompassing this investigation. These comparisons revealed no evidence of changes in the in vitro activities of the fluoroquinolones. In addition, the percentages of isolates in the three sampling periods for which MICs were above the resistance breakpoints were compared. Low percentages of resistant strains were detected, and there was no evidence of resistance rate changes over time. For example, by use of a ciprofloxacin MIC of [>=]4 {micro}g/ml to define resistance, the proportions of isolates from the three sampling periods for which MICs were at or above this breakpoint were 1.2, 1.6, and 1.4%, respectively. A total of 164 unique isolates (n = 58 from 1994-1995, 65 from 1997-1998, and 42 from 1999-2000) were examined for evidence of mutations in the quinolone resistance-determining regions (QRDRs) of the parC and the gyrA genes. Forty-nine isolates harbored at least one mutation in the QRDRs of one or both genes (1994-1995, n = 15; 1997-1998, n = 19; 1999-2000, n = 15). Among the 4,650 isolates of S. pneumoniae examined in the study, we estimated that 0.3% had mutations in both the parC and gyrA loci. The majority of mutations (67.3% of the mutations in 49 isolates with mutations) were amino acid substitutions in the parC locus only. Four isolates had a mutation in the gyrA locus only, and 12 isolates had mutations in both genes (8.2 and 24.5% of isolates with mutations, respectively). There was no significant difference in the number of isolates with parC and/or gyrA mutations detected during each study period. Finally, because of the magnitude of the study, we had reasonably large numbers of pneumococcal isolates with genotypically defined mechanisms of fluoroquinolone resistance and were thus able to determine the effects of specific resistance mutations on the activities of different fluoroquinolones. In general, isolates with mutations in parC only were resistant to ciprofloxacin but remained susceptible to levofloxacin, gatifloxacin, and moxifloxacin, whereas isolates with mutations in gyrA only and isolates with mutations in both parC and gyrA were resistant to all four fluoroquinolones tested.

Isoniazid is a first-line antibiotic used in the treatment of infections caused by Mycobacterium tuberculosis. Isoniazid is a prodrug requiring oxidative activation by the catalase-peroxidase hemoprotein, KatG. Resistance to isoniazid can be obtained by point mutations in the katG gene, with one of the most common being a threonine-for-serine substitution at position 315 (S315T). The S315T mutation is found in more than 50% of isoniazid-resistant clinical isolates and results in an ~200-fold increase in the MIC of isoniazid compared to that for M. tuberculosis H37Rv. In the present study we investigated the hypothesis that superoxide plays a role in KatG-mediated isoniazid activation. Plumbagin and clofazimine, compounds capable of generating superoxide anion, resulted in a lower MIC of isoniazid for M. tuberculosis H37Rv and a strain carrying the S315T mutation. These agents did not cause as great of an increase in isoniazid susceptibility in the mutant strain when the susceptibilities were assessed by using the inhibitory concentration that causes a 50% decrease in growth. These results provide evidence that superoxide can play a role in isoniazid activation. Since clofazimine alone has antitubercular activity, the observation of synergism between clofazimine and isoniazid raises the interesting possibility of using both drugs in combination to treat M. tuberculosis infections.

The development of novel antibacterial agents is decreasing despite increasing resistance to presently available agents among common pathogens. Insights into relationships between pharmacodynamics and resistance may provide ways to optimize the use of existing agents. The evolution of resistance was examined in two ciprofloxacin-susceptible Staphylococcus aureus strains exposed to in vitro-simulated clinical and experimental ciprofloxacin pharmacokinetic profiles for 96 h. As the average steady-state concentration (Cavg ss) increased, the rate of killing approached a maximum, and the rate of regrowth decreased. The enrichment of subpopulations with mutations in grlA and low-level ciprofloxacin resistance also varied depending on the pharmacokinetic environment. A regimen producing values for Cavg ss slightly above the MIC selected resistant variants with grlA mutations that did not evolve to higher levels of resistance. Clinical regimens which provided values for Cavg ss intermediate to the MIC and mutant prevention concentration (MPC) resulted in the emergence of subpopulations with gyrA mutations and higher levels of resistance. A regimen producing values for Cavg ss close to the MPC selected grlA mutants, but the appearance of subpopulations with higher levels of resistance was diminished. A regimen designed to maintain ciprofloxacin concentrations entirely above the MPC appeared to eradicate low-level resistant variants in the inoculum and prevent the emergence of higher levels of resistance. There was no relationship between the time that ciprofloxacin concentrations remained between the MIC and the MPC and the degree of resistance or the presence or type of ciprofloxacin-resistance mutations that appeared in grlA or gyrA. Regimens designed to eradicate low-level resistant variants in S. aureus populations may prevent the emergence of higher levels of fluoroquinolone resistance.

Mutations in the pfcr\(\text{t}\) and pfmdr1 genes have been associated with chloroquine resistance in Plasmodium falciparum. Ten and five mutations, respectively, have been identified in these genes from chloroquine-resistant parasites worldwide. Mutation patterns in pfcr\(\text{t}\) revealed that chloroquine resistance evolved independently in southeast Asia, South America, and Papua New Guinea. However, the evolution of chloroquine resistance in the rest of the Pacific region is unclear. In this study, we examined sequence polymorphisms in these genes in isolates from Morong, Philippines, and compared them to known chloroquine resistance sequences. Two novel mutations, A144T and L160Y, were identified outside of the 10 known mutations in pfcr\(\text{t}\) in Morong isolates. These novel mutations were identified only in parasites with K76T and N326D but without the common A220S mutation found in most chloroquine-resistant isolates. This represents a unique chloroquine resistance allelic type (K76T/A144T/L160Y/N326D) not previously found elsewhere in the world. One Morong isolate also had an additional C72S mutation, whereas only one isolate possessed an allelic type typical of chloroquine resistance in Asia. Parasites with the novel pfcr\(\text{t}\) allelic types were resistant to chloroquine in vitro and were unresponsive to verapamil (0.9 \(\mu\)M) chemosensitization, similar to chloroquine-resistant parasites from South America and Papua New Guinea. These results suggest that chloroquine resistance evolved independently in the Philippines and represents a second chloroquine resistance founder event in the South Pacific.


Three classes of macrolide resistance phenotypes and three different erythromycin resistance determinants were found among 127 erythromycin-resistant group A streptococcal (GAS) isolates recovered from 355 (35.8%) pediatric pharyngitis patients in Rome, Italy. According to emm and sof sequence typing results, erythromycin-resistant isolates comprised 11 different clonal types. Remarkably, 126 of the 127 macrolide-resistant isolates were serum opacity factor (sof) gene positive. These data suggest a strong association between macrolide resistance and the presence of sof among GAS isolates recovered from Italian pediatric pharyngitis patients.


Abacavir is a potent new carbocyclic nucleoside analogue. We employed our hollow-fiber pharmacodynamic modeling system to examine the antiretroviral effects of different abacavir exposures, as well as the impact of the schedule of drug administration on efficacy. Dose ranging of abacavir revealed that a concentration of four times the 50% effective concentration (EC50) (approximately the EC95) was required to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) (strain MN) either in a continuous-infusion hollow-fiber experiment or in a classical tissue culture flask experiment. In contrast to earlier work with another drug class (HIV-1 protease inhibitors), addition of physiological amounts of the human drug binding proteins albumin and \(\alpha\)1 acid glycoprotein revealed that there was little impact on the antiviral effect of the drug. Comparison of equivalent exposures (an area under the concentration-time curve [AUC] developed by approximately 500 mg per day of orally administered abacavir), either in a continuous-infusion mode or as a single oral dose of abacavir, demonstrated no difference in the
ability to suppress either strain IIIb or strain MN. Comparison of administration of 250 mg every 12 h (q12h) versus once-daily administration of 500 mg for strain MN again showed no significant difference in suppressive effect. These experiments were carried out over 8 to 15 days. Because of these promising initial results, we extended the experiment to 30 days and examined three different schedules of administration that generated the same AUC at 24 h (AUC24): 300 mg q12h, 600 mg q24h, and 1,200 mg q48h. The aim of the last of these regimens was to definitively demonstrate schedule failure. There was little difference between the 1,200-mg q48h treatment group and the untreated control at 30 days. Likewise, there was little difference between the 600-mg q24h and 300-mg q12h treatment groups. However, at circa day 18 of the experiment, there was a small increase in viral output of p24 in the once-daily dosing unit. Examination of virus from all groups demonstrated no phenotypic or genotypic differences. The small difference in hollow-fiber unit p24 in the once-daily dosing group was not due to emergence of resistance over the 30-day single-drug exposure. We conclude that the dose of abacavir currently being studied in clinical trials (300 mg orally q12h) will be efficacious for the majority of sensitive clinical isolates of HIV-1. These in vitro data also suggest that this drug may be able to be administered to patients on a once-daily basis at a dose of 600 mg.


http://aac.asm.org/cgi/content/abstract/46/8/2684

A PCR-based technique using molecular beacons was developed to detect the chloroquine resistance-associated pfcr7 K76T point mutation in Plasmodium falciparum. One hundred thirty African clinical isolates were tested by the new method in comparison with the PCR-restriction fragment length polymorphism method. This rapid and inexpensive genomic assay could expand the possibilities for monitoring chloroquine resistance.


http://aac.asm.org/cgi/content/abstract/49/1/366

Methicillin-resistant Staphylococcus aureus (MRSA) isolates have previously been classified into major epidemic clonal types by pulsed-field gel electrophoresis in combination with multilocus sequence typing (MLST) and staphylococcal cassette chromosome mec typing. We aimed to investigate whether genetic variability in potentially polymorphic domains of virulence-related factors could provide another level of differentiation in a diverse collection of epidemic MRSA clones. The target regions of strains representative of epidemic clones and genetically related methicillin-susceptible S. aureus isolates from the 1960s that were sequenced included the R domains of clfA and clfB; the D, W, and M regions of fnbA and fnbB; and three regions in the agr operon. Sequence variation ranged from very conserved regions, such as those for RNAIII and the agr interpromoter region, to the highly polymorphic R regions of the clf genes. The sequences of the clf R domains could be grouped into six major sequence types on the basis of the sequences in their 3' regions. Six sequence types were also observed for the fnb sequences at the amino acid level. From an evolutionary point of view, it was interesting that a small DNA stretch at the 3' clf R-domain sequence and the fnb sequences agreed with the results of MLST for this set of strains. In particular, clfB R-domain sequences, which had a high discriminatory capacity and with which the types distinguished were congruent with those obtained by other molecular typing methods, have potential for use for the typing of S. aureus. Clone- and strain-specific sequence motifs in the clf and fnb genes may represent useful additions to a typing
methodology with a DNA array.


http://aac.asm.org/cgi/content/abstract/46/2/350

Most Aeromonas strains isolated from two European rivers were previously found to be resistant to nalidixic acid. In order to elucidate the mechanism of this resistance, 20 strains of Aeromonas caviae (n = 10), A. hydrophila (n = 5), and A. sobria (n = 5) complexes, including 3 reference strains and 17 environmental isolates, were investigated. Fragments of the gyrA, gyrB, parC, and parE genes encompassing the quinolone resistance-determining regions (QRDRs) were amplified by PCR and sequenced. Results obtained for the six sensitive strains showed that the GyrA, GyrB, ParC, and ParE QRDR fragments of Aeromonas spp. were highly conserved ([≥]96.1% identity), despite some genetic polymorphism; they were most closely related to those of Vibrio spp., Pseudomonas spp., and members of the family Enterobacteriaceae (72.4 to 97.1% homology). All 14 environmental resistant strains carried a point mutation in the GyrA QRDR at codon 83, leading to the substitution Ser-83[gt]Ile (10 strains) or Ser-83[gt]Arg. In addition, seven strains harbored a mutation in the ParC QRDR either at position 80 (five strains), generating a Ser-80[gt]Ile (three strains) or Ser-80[gt]Arg change, or at position 84, yielding a Glu-84[gt]Lys modification. No amino acid alterations were discovered in the GyrB and ParE QRDRs. Double gyrA-parC missense mutations were associated with higher levels of quinolone resistance compared with the levels associated with single gyrA mutations. The most resistant strains probably had an additional mechanism(s) of resistance, such as decreased accumulation of the drugs. Our data suggest that, in mesophilic Aeromonas spp., as in other gram-negative bacteria, gyrase and topoisomerase IV are the primary and secondary targets for quinolones, respectively.


http://aac.asm.org/cgi/content/abstract/46/5/1410

A newly discovered gene, designated tcrB, which is located on a conjugative plasmid conferring acquired copper resistance in Enterococcus faecium, was identified in an isolate from a pig. The tcrB gene encodes a putative protein belonging to the Cpx-type ATPase family with homology (46%) to the CopB protein from Enterococcus hirae. The tcrB gene was found in E. faecium isolated from pigs (75%), broilers (34%), calves (16%), and humans (10%) but not in isolates from sheep. Resistant isolates, containing the tcrB gene, grew on brain heart infusion agar plates containing up to 28 mM CuSO4 compared to only 4 mM for the susceptible isolates. Copper resistance, and therefore the presence of the tcrB gene, was strongly correlated to macrolide and glycopeptide resistance in isolates from pigs, and the tcrB gene was shown to be located on the same conjugative plasmid as the genes responsible for resistance to these two antimicrobial agents. The frequent occurrence of this new copper resistance gene in isolates from pigs, where copper sulfate is being used in large amounts as feed additive, suggests that the use of copper has selected for resistance.

http://aac.asm.org/cgi/content/abstract/47/5/1514

Mutations in the dihydrofolate reductase (dhfr) genes of Plasmodium falciparum and P. vivax are associated with resistance to the antifolate antimalarial drugs. P. vivax dhfr sequences were obtained from 55 P. vivax isolates (isolates Belem and Sal 1, which are established lines originating from Latin America, and isolates from patient samples from Thailand [n = 44], India [n = 5], Iran [n = 2], and Madagascar [n = 2]) by direct sequencing of both strands of the purified PCR product and were compared to the P. vivax dhfr sequence from a P. vivax parasite isolated in Pakistan (isolate ARI/Pakistan), considered to represent the wild-type sequence. In total, 144 P. vivax dhfr mutations were found at only 12 positions, of which 4 have not been described previously. An F[\rightarrow]L mutation at residue 57 had been observed previously, but a novel codon (TTA) resulted in a mutation in seven of the nine mutated variant sequences. A new mutation at residue 117 resulted in S[\rightarrow]T (S[\rightarrow]N has been described previously). These two variants are the same as those observed in the P. falciparum dhfr gene at residue 108, where they are associated with different levels of antifolate resistance. Two novel mutations, I[\rightarrow]L at residue 13 and T[\rightarrow]M at residue 61, appear to be unique to P. vivax. The clinical, epidemiological, and sequence data suggest a sequential pathway for the acquisition of the P. vivax dhfr mutations. Mutations at residues 117 and 58 arise first when drug pressure is applied. Highly mutated genes carry the S[\rightarrow]T rather than the S[\rightarrow]N mutation at residue 117. Mutations at residues 57 and 61 then occur, followed by a fifth mutation at residue 13.


http://aac.asm.org/cgi/content/abstract/46/4/1086

In order to track the evolution of primary protease inhibitor (PI) resistance mutations in human immunodeficiency virus type 1 (HIV-1) isolates, baseline and follow-up protease sequences were obtained from patients undergoing salvage PI therapy who presented initially with isolates containing a single primary PI resistance mutation. Among 78 patients meeting study selection criteria, baseline primary PI resistance mutations included L90M (42% of patients), V82A/F/T (27%), D30N (21%), G48V (6%), and I84V (4%). Despite the switching of treatment to a new PI, primary PI resistance mutations present at the baseline persisted in 66 of 78 (85%) patients. D30N persisted less frequently than L90M (50% versus 100%, respectively; P < 0.001) and V82A/F/T (50% versus 81%, respectively; P = 0.05). HIV-1 isolates from 38 (49%) patients failing PI salvage therapy developed new primary PI resistance mutations including L90M, I84V, V82A, and G48V. Common combinations of primary and secondary PI resistance mutations after salvage therapy included mutations at amino acid positions 10, 82, and 46 and/or 54 in 16 patients; 10, 90, and 71 and/or 73 in 14 patients; 10, 73, 84, 90, and 46 and/or 54 in 5 patients; 10, 48, and 82 in 5 patients; and 30, 88 and 90 in 5 patients. In summary, during salvage PI therapy, most HIV-1 isolates with a single primary PI resistance mutation maintained their original mutations, and 49% developed additional primary PI resistance mutations. The persistence of L90M, V82A/F/T, G48V, and I84V during salvage therapy suggests that these mutations play a role in clinical resistance to multiple PIs.

It is generally thought that there is full cross-resistance in Mycobacterium tuberculosis between the aminoglycoside drugs kanamycin and amikacin. However, kanamycin resistance and amikacin susceptibility were seen in 43 of 79 (54%) multidrug-resistant Estonian isolates, indicating that there might be a need to test the resistance of M. tuberculosis isolates to both drugs.


Resistance to quinolone antibiotics has been associated with single-nucleotide polymorphisms (SNPs) in the quinolone resistance-determining region (QRDR) of gyrA. Mutations in the gyrA gene were compared by using mutant populations derived from wild-type Salmonella enterica serovar Enteritidis and its isogenic mutS:Tn10 mutator counterpart. Spontaneous mutants arising during nonselective growth were isolated by selection with either nalidixic acid, enrofloxacin, or ciprofloxacin. QRDR SNPs were identified in approximately 70% (512 of 695) of the isolates via colony hybridization with radiolabeled oligonucleotide probes. Notably, transition base substitution SNPs in the QRDR were dramatically increased in mutants derived from the mutS strain. Some, but not all, antibiotic-resistant mutants lacking QRDR SNPs were resistant to tetracycline and chloramphenicol, consistent with alterations in nonspecific efflux pumps or other membrane transport mechanisms. Changing the selection conditions shifted the mutation spectrum. Selection with ciprofloxacin was least likely to yield a mutant harboring either a QRDR SNP or chloramphenicol resistance. Selection with enrofloxacin was more likely to yield mutants containing Ser83[−→]Phe mutations, whereas selection with ciprofloxacin or nalidixic acid favored recovery of Asp87[−→]Gly mutants. Fluoroquinolone-resistant Salmonella strains isolated from veterinary or clinical settings frequently display a mutational spectrum with a preponderance of transition SNPs in the QRDR, the pattern found in vitro among mutS mutator mutants reported here. Both the preponderance of transition mutations and the varied mutation spectra reported for veterinary and clinical isolates suggest that bacterial mutators defective in methyl-directed mismatch repair may play a role in the emergence of quinolone and fluoroquinolone resistance in feral settings.


The genotypic inhibitory quotient (GIQ) has been proposed as a way to integrate drug exposure and genotypic resistance to protease inhibitors and can be useful to enhance the predictivity of virologic response for boosted protease inhibitors. The aim of this study was to evaluate the predictivity of the GIQ in 116 protease inhibitor-experienced patients treated with lopinavir-ritonavir. The overall decrease in human immunodeficiency virus type 1 (HIV-1) RNA from baseline to month 6 was a median of -1.50 log10 copies/ml and 40% of patients had plasma HIV-1 RNA below 400 copies/ml at month 6. The overall median lopinavir study-state Cmin concentration was 5,856 ng/ml. Using univariate linear regression analyses, both lopinavir GIQ and the number of baseline lopinavir mutations were highly associated with virologic response.
through 6 months. In the multivariate analysis, only lopinavir GIQ, baseline HIV RNA, and the number of prior protease inhibitors were significantly associated with response. When the analysis was limited to patients with more highly mutant viruses (three or more lopinavir mutations), only lopinavir GIQ remained significantly associated with virologic response. This study suggests that GIQ could be a better predictor of the virologic response than virological (genotype) or pharmacological (minimal plasma concentration) approaches used separately, especially among patients with at least three protease inhibitor resistance mutations. Therapeutic drug monitoring for patients treated by lopinavir-ritonavir would likely be most useful in patients with substantially resistant viruses.

http://aac.asm.org/cgi/content/abstract/47/2/594

Forty-nine protease inhibitor (PI)-experienced but amprenavir (APV)-naive patients experiencing virological failure were treated with ritonavir (RTV) (100 mg twice a day [b.i.d.]) plus APV (600 mg b.i.d.). Patients responded to therapy with a median viral load decrease of -1.32 log10 by week 12. The addition of low-dose RTV enhanced the minimal APV concentration in plasma (APV Cmin) up to 10-fold compared with that obtained with APV (1,200 mg b.i.d.) without RTV. Baseline PI resistance mutations (L10F/I/V, K20M/R, E35D, R41K, I54V, L63P, V82A/F/T/S, I84V) identified by univariate analysis and included in a genotypic score and APV Cmin at week 8 were predictive of the virological response at week 12. The response to APV plus RTV was significantly reduced in patients with six or more of the resistance mutations among the ones defined above. The genotypic inhibitory quotient, calculated as the ratio of the APV Cmin to the number of human immunodeficiency virus type 1 protease mutations, was a better predictor than the virological or pharmacological variables used alone. This genotypic inhibitory quotient could be used in therapeutic drug monitoring to define the concentrations in plasma needed to control replication of viruses with different levels of PI resistance, as measured by the number of PI resistance mutations.

http://aac.asm.org/cgi/content/abstract/48/4/1289

Rifampin is a major drug used in the treatment of tuberculosis infections, and increasing rifampin resistance represents a worldwide clinical problem. Resistance to rifampin is caused by mutations in the rpoB gene, encoding the (beta)-subunit of RNA polymerase. We examined the effect of three different rpoB mutations on the fitness of Mycobacterium tuberculosis. Rifampin-resistant mutants were isolated from a virulent clinical isolate of M. tuberculosis (strain Harlingen) in vitro at a mutation frequency of 2.3 x 10^-8. Mutations in the rpoB gene were identified, and the growth rates of three defined mutants were measured by competition with the susceptible parent strain in laboratory medium and by single cultures in a macrophage cell line and in laboratory medium. All of the mutants showed a decreased growth rate in the three assays. The relative fitness of the mutants varied between 0.29 and 0.96 (that of the susceptible strain was set to 1.0) depending on the specific mutant and assay system. Unexpectedly, the relative fitness ranking of the mutants differed between the different assays. In conclusion, rifampin resistance is associated with a cost that is conditional.

http://aac.asm.org/cgi/content/abstract/47/5/1760

We have developed a genetic system to monitor the activity of the hepatitis C virus (HCV) NS3 serine protease. This genetic system is based on the bacteriophage lambda regulatory circuit where the viral repressor cl is specifically cleaved to initiate the switch from lysogeny to lytic infection. An HCV protease-specific target, NS5A-5B, was inserted into the lambda phage cl repressor. The target specificity of the HCV NS5A-5B repressor was evaluated by coexpression of this repressor with a â€œgalactosidase (â€œgal-HCV NS32-181/421-34 protease construct. Upon infection of Escherichia coli cells containing the two plasmids encoding the cl.HCV5AB-cro and the â€œgal-HCV NS32-181/421-34 protease constructs, lambda phage replicated up to 8,000-fold more efficiently than in cells that did not express the HCV NS32-181/421-34 protease. This simple, rapid, and highly specific assay can be used to monitor the activity of the HCV NS3 serine protease, and it has the potential to be used for screening specific inhibitors.


http://aac.asm.org/cgi/content/abstract/48/8/2888

Soil bacteria are among the most prodigious producers of antibiotics. The Bacillus subtilis LiaRS (formerly YvqCE) two-component system is one of several antibiotic-sensing systems that coordinate the genetic response to cell wall-active antibiotics. Upon the addition of vancomycin or bacitracin, LiaRS autoregulates the liaIHGFSR operon. We have characterized the promoter of the lia operon and defined the cis-acting sequences necessary for antibiotic-inducible gene expression. A survey for compounds that act as inducers of the lia promoter revealed that it responds strongly to a subset of cell wall-active antibiotics that interfere with the lipid II cycle in the cytoplasmic membrane (bacitracin, nisin, ramoplanin, and vancomycin). Chemicals that perturb the cytoplasmic membrane, such as organic solvents, are also weak inducers. Thus, the reporter derived from PliaI (the liaI promoter) provides a tool for the detection and classification of antimicrobial compounds.


http://aac.asm.org/cgi/content/abstract/47/5/1658

Tropheryma whippelii, the agent of Whipple's disease, grows fastidiously only in cell cultures without plaque production, and only three strains have been passaged. The formation of bacterial clumps in the supernatant precludes enumeration of viable bacteria and MIC determination. We evaluated the bacteriostatic effects of fluoroquinolones against two T. whippelii isolates by measuring the inhibition of the DNA copy number increase by real-time quantitative PCR. The analysis of the T. whippelii genome database allowed the identification not only of the gyrA gene but also the parC gene encoding the alpha subunit of the natural fluoroquinolone targets DNA gyrase (GyrA) and topoisomerase IV (ParC), respectively. The parC gene was detected in
actinobacteria for the first time. High ciprofloxacin MICs (4 and 8 μg/ml) were correlated with the presence in T. whipplei GyrA and ParC sequences with an alanine residue at positions 83 and 80 (Escherichia coli numbering), respectively. Alanines at these positions have previously been associated with increased fluoroquinolone resistance in E. coli and mycobacteria. However, the MIC of levofloxacin was low (0.25 μg/ml). The same T. whipplei GyrA and ParC sequences were found in two other cultured strains and in nine uncultured tissue samples from Whipple's disease patients, allowing one to speculate that T. whipplei is naturally relatively resistant to fluoroquinolones.


http://aac.asm.org/cgi/content/abstract/47/5/1719

A collection of Aspergillus fumigatus mutants highly resistant to itraconazole (RIT) at 100 μg ml-1 were selected in vitro (following UV irradiation as a preliminary step) to investigate mechanisms of drug resistance in this clinically important pathogen. Eight of the RIT mutants were found to have a mutation at Gly54 (G54E, -K, or -R) in the azole target gene CYP51A. Primers designed for highly conserved regions of multidrug resistance (MDR) pumps were used in reverse transcriptase PCR amplification reactions to identify novel genes encoding potential MDR efflux pumps in A. fumigatus. Two genes, AfuMDR3 and AfuMDR4, showed prominent changes in expression levels in many RIT mutants and were characterized in more detail. Analysis of the deduced amino acid sequence encoded by AfuMDR3 revealed high similarity to major facilitator superfamily transporters, while AfuMDR4 was a typical member of the ATP-binding cassette superfamily. Real-time quantitative PCR with molecular beacon probes was used to assess expression levels of AfuMDR3 and AfuMDR4. Most RIT mutants showed either constitutive high-level expression of both genes or induction of expression upon exposure to itraconazole. Our results suggest that overexpression of one or both of these newly identified drug efflux pump genes of A. fumigatus and/or selection of drug target site mutations are linked to high-level itraconazole resistance and are mechanistic considerations for the emergence of clinical resistance to itraconazole.


http://aac.asm.org/cgi/content/abstract/46/3/925

A new natural TEM derivative, named TEM-87, was identified in a Proteus mirabilis isolate from an Italian hospital. Compared to TEM-1, TEM-87 contains the following mutations: E104K, R164C, and M182T. Kinetic analysis of TEM-87 revealed extended-spectrum activity against oxyimino cephalosporins (preferentially ceftazidime) and aztreonam. Expression of blaTEM-87 in Escherichia coli decreased the host susceptibility to these drugs.


http://aac.asm.org/cgi/content/abstract/48/7/2736
The nucleotide sequences of the pncA genes within 55 multidrug-resistant pyrazinamide-resistant Mycobacterium tuberculosis clinical isolates were determined. Fifty-three out of the 55 isolates were pyrazinamidase (PZase) negative. Four strains contained a wild-type pncA gene, and PZase activity was undetectable in two of these strains. Seven of the 18 identified pncA mutations found have not been described in previous studies.


http://aac.asm.org/cgi/content/abstract/49/2/488

To compare mutations in the DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes of Clostridium perfringens, which are associated with in vitro exposure to fluoroquinolones, resistant mutants were selected from eight strains by serial passage in the presence of increasing concentrations of norfloxacin, ciprofloxacin, gatifloxacin, or trovafloxacin. The nucleotide sequences of the entire gyrA, gyrB, parC, and parE genes of 42 mutants were determined. DNA gyrase was the primary target for each fluoroquinolone, and topoisomerase IV was the secondary target. Most mutations appeared in the quinolone resistance-determining regions of gyrA (resulting in changes of Asp-87 to Tyr or Gly-81 to Cys) and parC (resulting in changes of Asp-93 or Asp-88 to Tyr or Ser-89 to Ile); only two mutations were found in gyrB, and only two mutations were found in parE. More mutants with multiple gyrA and parC mutations were produced with gatifloxacin than with the other fluoroquinolones tested. Allelic diversity was observed among the resistant mutants, for which the drug MICs increased 2- to 256-fold. Both the structures of the drugs and their concentrations influenced the selection of mutants.


http://aac.asm.org/cgi/content/abstract/46/2/590

Mycoplasma gallisepticum enrofloxacin-resistant mutants were generated by stepwise selection in increasing concentrations of enrofloxacin. Alterations were found in the quinolone resistance-determining regions of the four target genes encoding DNA gyrase and topoisomerase IV from these mutants. This is the first description of such mutations in an animal mycoplasma species.


http://aac.asm.org/cgi/content/abstract/49/1/444

In this study the nucleotide sequence of the pncA gene from 59 Mycobacterium tuberculosis clinical isolates was analyzed. Mutations in the pncA gene were identified in 29 of 40 pyrazinamide-resistant isolates, and no pyrazinamidase activity was detected in 39 of them. Twelve mutations found in this work have not been described previously.
Tigecycline is an expanded broad-spectrum antibacterial agent that is active against many clinically relevant species of bacterial pathogens, including Klebsiella pneumoniae. The majority of K. pneumoniae isolates are fully susceptible to tigecycline; however, a few strains that have decreased susceptibility have been isolated. One isolate, G340 (for which the tigecycline MIC is 4 \( \mu \)g/ml and which displays a multidrug resistance [MDR] phenotype), was selected for analysis of the mechanism for this decreased susceptibility by use of transposon mutagenesis with IS903\( \phi \)-kan. A tigecycline-susceptible mutant of G340, GC7535, was obtained (tigecycline MIC, 0.25 \( \mu \)g/ml). Analysis of the transposon insertion mapped it to ramA, a gene that was previously identified to be involved in MDR in K. pneumoniae. For GC7535, the disruption of ramA led to a 16-fold decrease in the MIC of tigecycline and also a suppression of MDR. Trans-complementation with plasmid-borne ramA restored the original parental phenotype of decreased susceptibility to tigecycline. Northern blot analysis revealed a constitutive overexpression of ramA that correlated with an increased expression of the AcrAB transporter in G340 compared to that in tigecycline-susceptible strains. Laboratory mutants of K. pneumoniae with decreased susceptibility to tigecycline could be selected at a frequency of approximately 4 x 10-8. These results suggest that ramA is associated with decreased tigecycline susceptibility in K. pneumoniae due to its role in the expression of the AcrAB multidrug efflux pump.

Sixteen isolates of Enterococcus faecalis were recovered from retail poultry samples (seven chickens and nine turkeys) purchased from grocery stores in the greater Washington, D.C., area. PCR for known streptogramin resistance genes identified vat(E) in five E. faecalis isolates (three isolates from chickens and two isolates from turkeys). The vat(E) gene was transmissible on a ca. 70-kb plasmid, along with resistance to erythromycin, tetracycline, and streptomycin, by conjugation to E. faecalis and Enterococcus faecium recipient strains. DNA sequencing showed little variation between E. faecalis vat(E) genes from the chicken samples; however, one E. faecalis vat(E) gene from a turkey sample possessed 5 nucleotide changes that resulted in four amino acid substitutions. None of these substitutions in the vat(E) allele have previously been described. This is the first report of vat(E) in E. faecalis and its transferability to E. faecium, which indicates that E. faecalis can act as a reservoir for the dissemination of vat(E)-mediated streptogramin resistance to E. faecium.

We studied 20 Chlamydia pneumoniae isolates obtained from respiratory sites and atheroma tissue of patients from various geographic areas to determine the susceptibilities of these isolates.
to a new des-fluoroquinolone, garenoxacin, and to levofloxacin. In addition, we assessed the cultures with these isolates by PCR for the presence or absence of Mycoplasma sp. DNA. Both the MIC at which 90% of isolates are inhibited (MIC90) and the minimal bactericidal concentration at which 90% of isolates are killed (MBC90) for garenoxacin were 0.06 (micro)g/ml, and both the MIC90 and the MBC90 for levofloxacin were 2.0 (micro)g/ml. The activity of garenoxacin against C. pneumoniae was 32-fold greater than that of levofloxacin. Mycoplasma sp. DNA was detected by PCR in 17 of 20 cultures. Mycoplasma amplicons from five Mycoplasma DNA-positive C. pneumoniae cultures were sequenced and found to represent four Mycoplasma species. Our data demonstrate that C. pneumoniae cultures frequently contain Mycoplasma DNA and that its presence in C. pneumoniae cultures does not appear to affect the susceptibility results for the two fluoroquinolones that we tested.


http://aac.asm.org/cgi/content/abstract/46/1/196

An Italian nationwide survey was carried out to assess the prevalences and the antimicrobial susceptibilities of members of the family Enterobacteriaceae producing extended-spectrum (beta)-lactamases (ESBLs). Over a 6-month period, 8,015 isolates were obtained from hospitalized patients and screened for resistance to extended-spectrum cephalosporins and monobactams. On the basis of a synergistic effect between clavulanate and selected (beta)-lactams (ceftazidime, aztreonam, cefotaxime, cefepime, and ceftriaxone), 509 isolates were found to be ESBL positive (6.3%). Colony blot hybridization with blaTEM and blaSHV DNA probes allowed one to distinguish four different genotypes: TEM-positive, SHV-positive, TEM-and SHV-positive, and non-TEM, non-SHV ESBL types. MICs for each isolate (E-test) were obtained for widely used (beta)-lactams, combinations of (beta)-lactams with (beta)-lactamase inhibitors, aminoglycosides, and fluoroquinolones. Among ESBL-positive strains, Klebsiella pneumoniae, Proteus mirabilis, and Escherichia coli accounted for 73.6% of isolates. Overall, TEM-type ESBLs were more prevalent than SHV-type enzymes (234 versus 173), whereas the prevalence of strains producing both TEM- and SHV-type ESBLs was similar to that of isolates producing non-TEM, non-SHV enzymes (55 and 38, respectively). In vitro, all but one of the ESBL-producing isolates remained susceptible to imipenem. Susceptibility to other drugs varied: piperacillin-tazobactam, 91%; amoxicillin-clavulanic acid, 85%; cefoxitin, 78%; amikacin, 76%; ampicillin-sulbactam, 61%; ciprofloxacin, 58%; and gentamicin, 56%. Associated resistance to aminoglycosides and ciprofloxacin was observed most frequently among TEM-positive strains. Since therapeutic options for multiresistant Enterobacteriaceae are limited, combinations of (beta)-lactams and (beta)-lactamase inhibitors appear to represent an important alternative for treating infections caused by ESBL-producing Enterobacteriaceae.


http://aac.asm.org/cgi/content/abstract/49/1/170

The knowledge of the effects of antimicrobial agents on the normal vaginal microflora is limited. The objective of the present study was to study the ecological impact of pivmecillinam on the normal vaginal microflora. In 20 healthy women, the estimated day of ovulation was determined during three subsequent menstrual cycles. Microbiological and clinical examinations were performed on the estimated day of ovulation and on day 3 in all cycles and also on day 7 after ovulation in cycles 1 and 2. Anaerobic and facultative anaerobic gram-positive rods, mainly
species of lactobacilli and actinomycetes, dominated the microflora. One woman was colonized on the third day of administration with a resistant Escherichia coli strain, and Candida albicans was detected in one woman on days 3 and 7 in cycle 2. No other major changes in the normal microflora occurred during the study. Administration of pivmecillinam had a minor ecological impact on the normal vaginal microflora.


http://aac.asm.org/cgi/content/abstract/49/5/2015

Plasma-derived sequences of human immunodeficiency virus type 1 (HIV-1) protease from 1,162 patients (457 drug-naive patients and 705 patients receiving protease inhibitor [PI]-containing antiretroviral regimens) led to the identification and characterization of 17 novel protease mutations potentially associated with resistance to PIs. Fourteen mutations were positively associated with PIs and significantly correlated in pairs and/or clusters with known PI resistance mutations, suggesting their contribution to PI resistance. In particular, E34Q, K43T, and K55R, which were associated with lopinavir treatment, correlated with mutations associated with lopinavir resistance (E34Q with either L33F or F53L, or K43T with I54A) or clustered with multi-PI resistance mutations (K43T with V62A and I54V or V82A, V32I, and I47V, or K55R with V82A, I54V, and M46I). On the other hand, C95F, which was associated with treatment with saquinavir and indinavir, was highly expressed in clusters with either L90M and I93L or V82A and G48V. K45R and K20T, which were associated with nelfinavir treatment, were specifically associated with D30N and N88D and with L90M, respectively. Structural analysis showed that several correlated positions were within 8 Å of each other, confirming the role of the local environment for interactions among mutations. We also identified three protease mutations (T12A, L63Q, and H69N) whose frequencies significantly decreased in PI-treated patients compared with that in drug-naive patients. They never showed positive correlations with PI resistance mutations; if anything, H69N showed a negative correlation with the compensatory mutations M36I and L101I. These mutations may prevent the appearance of PI resistance mutations, thus increasing the genetic barrier to PI resistance. Overall, our study contributes to a better definition of protease mutational patterns that regulate PI resistance and strongly suggests that other (novel) mutations beyond those currently known to confer resistance should be taken into account to better predict resistance to antiretroviral drugs.


http://aac.asm.org/cgi/content/abstract/48/9/3260

Most antimicrobial peptides (AMPs) impair the viability of target bacteria by permeabilizing bacterial membranes. However, the proline-rich AMPs have been shown to kill susceptible organisms without causing significant membrane perturbation and may act by inhibiting the activity of bacterial targets. To gain initial insight into the events that follow interaction of a proline-rich peptide with bacterial cells, we used DNA macroarray technology to monitor transcriptional alterations of Escherichia coli in response to challenge with a subinhibitory concentration of the proline-rich Bac7(1-35). Substantial changes in the expression levels of 70 bacterial genes from various functional categories were detected. Among these, 26 genes showed decreased expression, while 44 genes, including genes that are potentially involved in bacterial resistance to antimicrobials, showed increased expression. The generation of a transcriptional response under
the experimental conditions used is consistent with the ability of Bac7(1-35) to interact with bacterial components and affect biological processes in this organism.


http://aac.asm.org/cgi/content/abstract/47/9/2732

The first outbreak of multidrug-resistant (MDR) typhoid fever in Vietnam was in 1993, and by 1995 nearly 90% of cases were MDR. Plasmid HCM1, sequenced in full, is an incHI1 plasmid from Salmonella enterica serovar Typhi strain CT18, isolated in Vietnam in 1993. Restriction analysis shows that pHCM1 shares a restriction fragment length polymorphism (RFLP) pattern with plasmids isolated from the first outbreak and 10 of 17 MDR plasmids isolated from sporadic cases occurring at the same time in Vietnam. A core region of pHCM1 has significant DNA sequence similarity to plasmid R27, isolated in 1961 from S. enterica in the United Kingdom. There are five regions of DNA in pHCM1 which are not present in R27. Two of these are putative acquisition regions; the largest is 34.955 kbp in length and includes sequences of several antibiotic resistance genes and several insertion sequences. The borders of this region are defined by two identical IS10 left elements, associated with an inversion of DNA or with a truncated Tn10 element. The second, smaller region is 14.751 kbp and carries a trimethoprim resistance gene dfr4A cassette associated with a class 1 integrase. In 1993 to 1994, restriction analysis revealed some variations in the structures of Salmonella serovar Typhi MDR plasmids which were mapped to the two putative acquisition regions and three smaller variable regions. In 1996 a single RFLP type, RFLP7, was found to carry the dfrA7 and sul-1 genes, which were not present on R27 or pHCM1. This plasmid type appears to have a selective advantage over other plasmids with the same resistance phenotype.


http://aac.asm.org/cgi/content/abstract/46/8/2582

Mutations associated with fluoroquinolone resistance in clinical isolates of Proteus mirabilis were determined by genetic analysis of the quinolone resistance-determining region (QRDR) of gyrA, gyrB, parC, and parE. This study included the P. mirabilis type strain ATCC 29906 and 29 clinical isolates with reduced susceptibility (MIC, 0.5 to 2 (micro)g/ml) or resistance (MIC, [>=]4 (micro)g/ml) to ciprofloxacin. Susceptibility profiles for ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin were correlated with amino acid changes in the QRDRs. Decreased susceptibility and resistance were associated with double mutations involving both gyrA (S83R or -I) and parC (S80R or -I). Among these double mutants, MICs of ciprofloxacin varied from 1 to 16 (micro)g/ml, indicating that additional factors, such as drug efflux or porin changes, also contribute to the level of resistance. For ParE, a single conservative change of V364I was detected in seven strains. An unexpected result was the association of gyrB mutations with high-level resistance to fluoroquinolones in 12 of 20 ciprofloxacin-resistant isolates. Changes in GyrB included S464Y (six isolates), S464F (three isolates), and E466D (two isolates). A three-nucleotide insertion, resulting in an additional lysine residue between K455 and A456, was detected in gyrB of one strain. Unlike any other bacterial species analyzed to date, mutation of gyrB appears to be a frequent event in the acquisition of fluoroquinolone resistance among clinical isolates of P. mirabilis.

http://aac.asm.org/cgi/content/abstract/46/4/1098

Klebsiella pneumoniae isolates from Taiwan medical centers (50 strains; 1998 to 2000) with a CTX-M resistance phenotype (ceftazidime susceptible and ceftriaxone or cefotaxime nonsusceptible) were selected for initial isoelectric focusing analysis. \(\beta\)-Lactamases with pIs of 7.9 \((n = 22)\) and 8.4 \((n = 28)\) in addition to 5.4 and/or 7.6 were detected. DNA gene sequencing identified the \(\beta\)-lactamases with pIs of 7.9 and 8.4 as CTX-M-14 and CTX-M-3, respectively. Molecular typing suggested inter- and intrahospital clonal dissemination of these Taiwanese CTX-M-producing Klebsiella strains.


http://aac.asm.org/cgi/content/abstract/46/12/3900

Laboratory strains of Mycosphaerella graminicola with decreased susceptibilities to the azole antifungal agent cyproconazole showed a multidrug resistance phenotype by exhibiting cross-resistance to an unrelated chemical, cycloheximide or rhodamine 6G, or both. Decreased azole susceptibility was found to be associated with either decreased or increased levels of accumulation of cyproconazole. No specific relationship could be observed between azole susceptibility and the expression of ATP-binding cassette (ABC) transporter genes MgAtr1 to MgAtr5 and the sterol P450 14(a)-demethylase gene, CYP51. ABC transporter MgAtr1 was identified as a determinant in azole susceptibility since heterologous expression of the protein reduced the azole susceptibility of Saccharomyces cerevisiae and disruption of MgAtr1 in one specific M. graminicola laboratory strain with constitutive MgAtr1 overexpression restored the level of susceptibility to cyproconazole to wild-type levels. However, the level of accumulation in the mutant with an MgAtr1 disruption did not revert to the wild-type level. We propose that variations in azole susceptibility in laboratory strains of M. graminicola are mediated by multiple mechanisms.

**Antiviral Research** (19)


http://www.sciencedirect.com/science/article/B6T2H-476MP7D-7/2/c7ad6e137668c707ff6c306070ebde34

We attempted to detect drug-related HIV-1 pol gene mutations by selective polymerase chain reaction (PCR) using both proviral DNA and viral RNA isolated from patients (pts) with AIDS or
ARC receiving antiretroviral therapy. Peripheral blood mononuclear cell (PBM)-associated proviral DNA and serum-derived viral RNA were obtained from eight patients before and after receiving an alternating regimen of AZT and ddC for 15-41 months or ddI monotherapy for 12-26 months. These specimens were examined for the presence of mutations at positions 70, 74, 215 and 219. We noted that selective PCR results can be ambiguous depending on the quantity of DNA template employed. We, therefore, used the minimal quantity of DNA templates that yielded evaluable PCR products in this study. For all the eight pairs of pre- and post-therapy proviral DNA samples, selective PCR results agreed with independently determined nucleotide sequences. Results of reverse transcription of serum-derived viral RNA followed by selective PCR differed in some cases from those using the proviral DNA. In particular, the use of serum viral RNA appeared to allow earlier detection of changes in drug-related mutations than the use of PBM-associated proviral DNA. We conclude that (i) selective PCR using the minimum and sufficient number of PBM-associated proviral DNA and serum viral RNA copies successfully detects the presence of known pol gene mutations; (ii) drug-related mutations may be distinguished earlier in virions in serum (or plasma) than in proviral DNA in PBM; and (iii) quantification of HIV-1 prior to selective PCR may be an important component in monitoring the therapy of HIV-1 infection.


http://www.sciencedirect.com/science/article/B6T2H-4FCRC79-1/2/7d371194ec42a72a6ebe978b988f2049

Chronic hepatitis B virus (HBV) infection is endemic in Asia and its consequences are among the major public health problems in the world. Unfortunately, the therapeutic efficacies of present strategies are still unsatisfactory with a major concern about viral mutation. In search of effective antiviral agent, we examined the efficacy of extracts of Polygonum cuspidatum Sieb. et Zucc. (P. cuspidatum) against HBV in HepG2 2.2.15 cells by quantitative real time polymerase chain reaction. The expressions of viral antigens, HBeAg and HBsAg, were also determined by enzyme linked immunosorbent assay. The ethanol extract of P. cuspidatum could inhibit dose-dependently the production of HBV (p P. cuspidatum might also inhibit the production of HBV at a higher dosage. The expression of HBsAg was significantly increased by both ethanol extract and water extract of P. cuspidatum dose-dependently (p P. cuspidatum (30 [mu]g/ml) could inhibit the expression of HBeAg (p P. cuspidatum might contain compounds that would contribute to the control of HBV infection in the future. However, its promoting effect on the expression of HBsAg and its cytotoxicity should be monitored. Further purification of the active compounds, identification and modification of their structures to improve the efficacy and decrease the cytotoxicity are required.


http://www.sciencedirect.com/science/article/B6T2H-476MP7D-4/2/423fc428ad10b7f5c0b60a468a00a3b

In this study we compared how effectively the proinflammatory cytokines TNF-[alpha] and IFN-[beta] could inhibit HSV-1 replication in human corneal tissue fragments and monolayers of epithelial cells and fibroblasts derived from intact corneas, and investigated the mechanism responsible for the inhibition. Pretreatment of corneal tissue or cells derived therefrom with TNF-[alpha] (50 U/ml) and IFN-[gamma] (5 IU/ml) consistently induced a synergistic antiviral effect. Inhibition of HSV-1 growth was most evident in fibroblasts (> 1000-fold reduction) and less
apparent (7-25-fold reduction) when epithelial cells were the target. Virus suppression was correlated with the induction of IFN-[beta] because antibody to this cytokine but not IFN-[alpha] abrogated synergism. The more modest synergistic effect in epithelial cells was associated with a >= 4-fold reduction in the synthesis of IFN-[beta] protein and mRNA, and decreased responsiveness of these cells to the antiviral effect of IFN-[beta]. We conclude that the combination of TNF-[alpha] and IFN-[gamma] induces a synergistic antiviral effect in corneal cells. The degree of synergism observed varies with the corneal cell type, and is correlated with the amount of IFN-[beta] induced and the target cell responsiveness to the antiviral action of this cytokine.


http://www.sciencedirect.com/science/article/B6T2H-44NM21X-5/2/033bd8c1ba05fd1bf40ae2f80477e8f

The present study describes a novel meshing procedure that provided successful low-risk papillomavirus propagation and reproducible wart induction in human foreskin xenografts. The initial HPV6 and/or 11 inocula were collected from clinically excised human wart tissues and confirmed to be free of HPV16, 18 and 31 by PCR analysis. Human foreskin grafts were collected from a circumcision clinic, and pre-inoculated with HPV virions by scarification. Meshing was carried out with a Zimmer Skin Grafter Mesher. Grafts were cut to appropriate size (1 cm X 1 cm or 5 mm X 5 mm) for cutaneous or subcutaneous grafting to NIH-nu-bg-xid mice under halothane anesthesia. Cutaneous xenografts were dressed with antibiotics and protective band-aids for 3 weeks. In the paralleled experiment using the same viral stock containing both HPV6 and 11, and matched grafts, no visible papillomas were observed in non-meshed cutaneous xenografts (n = 4 up to 6 months). In comparison, six of eight cutaneous xenografts treated with the meshing procedure formed visible papillomas within 4 months. This high frequency of distinct papilloma induction over the surface of meshed xenografts were reproduced in subsequent experiments with viral stocks containing both HPV11 and 6 (8 out of 10 grafts), or with a single-type HPV11 inoculum (80-100%). In contrast, an initial viral stock of single-type HPV6 provided lower frequency and more delayed papilloma induction. Serial passage of HPV6 in the meshed xenograft appeared to improve both the induction frequency and growth rate up to the 3rd generation. Histology, in situ hybridization, and immunohistochemical analysis revealed similarity of xenograft warts to those observed in the clinic. The highly reproducible papilloma induction rate and successful viral stock propagation associated with the meshing procedure provide a novel feature in the HPV xenograft model.


http://www.sciencedirect.com/science/article/B6T2H-44NM21X-5/2/033bd8c1ba05fd1bf40ae2f80477e8f

The biological evaluation of mononucleotide prodrugs (pronucleotides) of various nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT), zalcitabine (ddC) and lamivudine (3TC) was reported in human T-lymphoid MOLT-4/8 cells which were grown continuously for more than 1 year in a medium containing cytarabine (Ara-C). In this cell line, expression of deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) was decreased in comparison to parental cells (3.8 and 2.9-fold, respectively). The lower mRNA level of TK1 correlated significantly with lower enzyme activity, whereas no dCK activity was detectable. In
Ara-C-resistant cells, anti-HIV-1 effects of ddC, 3TC and AZT were more than 100-fold lower compared with parental cells. In contrast, the corresponding mononucleoside phosphodiesters bearing S-acyl-2-thioethyl (SATE) groups as biolabile phosphate protection retained anti-HIV-1 activity due to their ability to bypass the first monophosphorylation step catalyzed by dCK or TK1. The results demonstrate that in vitro selection of T-lymphoid cells in the presence of Ara-C results in cross-resistance to deoxycytidine (ddC, 3TC) and thymidine (AZT) analogs and that these cellular resistance mechanisms can be bypassed by the use of bis(SATE) pronucleotides.


Persistent infections with a cardiotropic enterovirus, e.g. coxsackievirus B2 (CVB2), cause chronic myocarditis and eventually congestive heart failure. Therefore, the antiviral activity of WIN 54954, a capsid binding antiviral agent that inhibits enterovirus uncoating, was studied in persistently CVB2-infected cultures of human myocardial fibroblasts. Cultures displayed a typical carrier state infection with virus titers of 3.9+/−1.6 x 10⁵ plaque forming units (PFU)/ml and 0.99% infected cells. WIN 54954 (0.025-1 [μg/ml] application was started 7 days after infection of the cultures. Compared to the WIN 54954 concentration resulting in a 90% plaque number reduction (EC₉₀=0.197 [μg/ml] in acutely infected Vero cells, WIN 54954 reduced virus yields of myocardial fibroblast cultures more efficiently, e.g. more than 100 fold (99%) with 0.025 [μg/ml after 4 days of application. Antiviral effects of WIN 54954 increased with application time and at 0.025 [μg/ml WIN 54954 completely inhibited infectious virus progeny after 16 days. Increasing the WIN 54954 concentration up to 1 [μg/ml did not cause a greater inhibition of virus replication. In situ hybridization demonstrated that at 0.1 [μg/ml WIN 54954 reduced the number of infected cells from 0.99 to 0.18%, although a complete eradication of CVB2-infected cells was not achieved at concentrations as high as 1 [μg/ml. In conclusion, the results indicate that low concentrations of WIN 54954 are effective in treating persistent enterovirus infections of myocardial fibroblasts, although a complete eradication of the infection is not achieved with WIN 54954 as a single antiviral agent.


Oseltamivir carboxylate is a potent and specific inhibitor of influenza A and B neuraminidase (NA). Oseltamivir phosphate, the ethyl ester prodrug of oseltamivir carboxylate, is the first orally active NA inhibitor available for the prophylaxis and treatment of influenza A and B. It offers an improvement over amantadine and rimantadine which are active only against influenza A and rapidly generate resistant virus. The emergence of virus resistant to oseltamivir carboxylate in the treatment of naturally acquired influenza infection is low (about 1%). The types of NA mutation to arise are sub-type specific and largely predicted from in vitro drug selection studies. A substitution of the conserved histidine at position 274 for tyrosine in the NA active site has been selected via site directed mutagenesis, serial passage in culture under drug pressure in H1N1 and during the treatment of experimental H1N1 infection in man. Virus carrying H274Y NA enzyme selected in vivo has reduced sensitivity to oseltamivir carboxylate. The replicative ability in cell culture was reduced up to 3 logs, as was infectivity in animal models of influenza virus infection. Additionally,
The pathogenicity of the mutant virus is significantly compromised in ferret, compared to the corresponding wild type virus. Virus carrying a H274Y mutation is unlikely to be of clinical consequence in man.


http://www.sciencedirect.com/science/article/B6T2H-3YGV2M7-S/2/828da9e15bdbece5a05a2af540c21870

The HIV-1 protease (PR) is essential for the production of mature virions. As such, it has become a target for the development of anti-HIV chemotherapeutics. Multiple passages of virus in cell culture in the presence of PR inhibitors have resulted in the selection of variants with decreased sensitivity to inhibitors of the PR. The most common alteration observed is a single amino acid change at position 82. This particular position has been well characterized by several laboratories as being important for the susceptibility of the virus to inhibitors of PR function. Mutations which result in the substitution of the wild-type valine with alanine, phenylalanine, threonine or isoleucine at position 82 of the PR have been associated with decreased sensitivity to several PR inhibitors. We describe here a clinical strain of HIV-1 that contains an isoleucine at position 82 of the PR instead of the usual valine. This strain is unique in that it was isolated from a patient that was anti-retroviral naive, and in the past, variants at position 82 of the PR have only been found after treatment of patients or cell culture with PR inhibitors. Moreover, this virus remains sensitive to PR inhibitors of the cyclic urea and C-2 symmetrical diol classes.


http://www.sciencedirect.com/science/article/B6T2H-3YGTTBB-14/2/1adea0125b229e7e0f6e5f35130596e

We have analyzed mutations in the thymidine kinase (TK) gene of varicella zoster virus (VZV) which showed resistance to 5-iodo-2'-deoxyuridine (IDU) and 5-bromo-2'-deoxyuridine (BrDU). Through sequencing of the TK gene, we found three amino acids were exchanged (41 Asn -> Ser, 266 Cys -> Ile, 288 Ser -> Leu). These mutations were not located at either the nucleoside- or the ATP-binding site. This result suggests that the resistance to IDU and BrDU in this particular strain is due to the change in conformation of TK rather than the replacement of amino acids in the binding sites.


http://www.sciencedirect.com/science/article/B6T2H-46W05D2-4/2/26c6d158754c9852d647787aae9f3138

Recently, the use of the ribonucleotide reductase (RR) inhibitor hydroxyurea (HU) in combination with nucleoside analogs has gained attention as a potential strategy for anti-HIV-1 therapy.
However, appeal for the long-term use of HU in HIV-1 infection may be limited by its propensity to induce hematopoietic toxicity. We report a comparison of the efficacy and bone marrow toxicity of HU (400 and 200 mg/kg/day) with the novel RR inhibitors and free radical-scavenging compounds didox (DX; 3,4-dihydroxybenzohydroxamic acid; 350 mg/kg/day) and trimidox (TX; 3,4,5-trihydroxybenzamidoxime; 175 mg/kg/day) in the murine AIDS (LPBM5 MuLV) model of retrovirus infection. Infected mice received daily drug treatment for 8 weeks. Efficacy was determined by measuring drug effects on retroviral-induced disease progression (i.e. development of splenomegaly and hypergammaglobulinemia) and by evaluating splenic levels of proviral DNA. Bone marrow toxicity was evaluated by measuring peripheral blood indices (WBC, hematocrit and reticulocyte counts), femoral cellularity and by determining the numbers of hematopoietic progenitor cells (CFU-GM, BFU-E) per femur and spleen. Compared to infected controls receiving no drug treatment, disease progression was significantly suppressed by TX, DX and HU. However, HU was associated with mortality and induced significant hematopoietic toxicity in a time- and dose-dependent manner. Conversely, TX and DX effectively inhibited retrovirus-induced disease but did not induce hematopoietic toxicity. These results suggest that due to their reduced hematopoietic toxicity and ability to inhibit disease progression in murine AIDS, TX and DX may offer effective alternatives to HU therapy in HIV-1 infection.


http://www.sciencedirect.com/science/article/B6T2H-3YHG1HV-7/2/b9d2ec457e34932e623b3733751ddccf

The in vitro antiviral activity of a new series of cycloSal-pro-nucleotides derived from the acyclic nucleoside analogues aciclovir and penciclovir against herpes simplex virus type 1 (HSV-1), thymidine kinase deficient (TK-) HSV-1, and Epstein-Barr virus (EBV) was evaluated. Using the XTT-based tetrazolium reduction assay EZ4U, the cycloSal derivatives were examined for their antiviral and cytotoxic effects in HSV-1 as well as HSV-1-TK--infected Vero cells. The anti-EBV activity was assessed by means of an EBV DNA hybridization assay using a digoxigenin-labeled probe specific for the Bam H1-W-fragment of the EBV genome and by measuring viral capsid antigen (VCA) expression in P3HR-1 cells by indirect immunofluorescence. Among the new cycloSal-phosphotriesters the three aciclovir monophosphates proved to be potent and selective inhibitors of HSV-1 replication, EBV DNA synthesis and EB-VCA expression. Of interest is the retention of activity of the aciclovir monophosphates in HSV-1-TK--infected cells. Particularly 3-methyl-cycloSal-aciclovir monophosphate retained the same effectiveness, as compared to the wild type virus strain. In contrast to the aciclovir pro-nucleotides the penciclovir cycloSal-phosphotriesters exhibited at best only a marginal antiviral effect on HSV and EBV replication.


http://www.sciencedirect.com/science/article/B6T2H-3YW3WPMY-3/2/70a08fc2ab2b212eac28537e45570992

The reduction in the efficacy of rescue treatment (administered on a clinical basis) due to drug resistance was retrospectively quantified in 55 human immunodeficiency virus type 1 (HIV-1)-infected patients failing highly active antiretroviral therapy (HAART) by using a novel score calculation system based upon HIV-1 reverse transcriptase (RT) and protease (PR) mutations. Patients were all naive for nelfinavir (NFV) and efavirenz (EFV) and were assigned to one of the following rescue therapy schedules: (i) 17 patients received NFV+EFV+stavudine (d4T) (group
A); (ii) 14 patients received NFV+saquinavir (SQV)+lamivudine (3TC)+d4T/zidovudine (AZT) (group B); (iii) 19 patients received NFV+d4T+didanosine (ddI)/3TC/zalcitabine (ddC) (group C); (iv) five patients received miscellaneous treatments including NFV (group D). Responders were considered patients showing a drop in HIV-1 RNA level >0.5 log10 after 3 months of therapy. Forty-eight (28 responders and 20 non-responders) out of 55 patients completed the first 3 months of rescue therapy and reduction in HIV-1 viral load was found to be significantly higher in group A compared to groups B and C (81.2% responders vs. 38.5 and 40.0%, respectively). At baseline, no patient carried EFV- or d4T-resistant HIV-1 strains, despite prolonged administration of d4T, while 41/48 (87.2%) patients had mutations conferring resistance to NFV in the absence of previous treatment with this drug. A significant inverse correlation between reduction in viral load and reduction in therapy efficacy due to drug resistance, as determined by the score calculation system, was found (r=0.62). A cut-off value of 36% reduction in therapy efficacy showed a positive predictive value (capacity to detect failure of rescue treatment) of 81.2% and a negative predictive value (ability to detect successful treatment) of 75.8%. In addition, 45 out of 48 patients completed also the 9-12 month period of rescue therapy and 10/28 responders had a rebound in HIV-1 viral load level detected after the first 3 months of rescue therapy. Of these, 5/7 (71.4%) showed a further reduction in rescue therapy efficacy due to the emergence of new mutations.


http://www.sciencedirect.com/science/article/B6T2H-4F6K73J-1/2/77c5132a2b2915009b9012269c26e1bc

Highly active antiretroviral therapy (HAART) is unlikely to affect reservoirs of HIV in latently infected cells. Anti-gene compounds, such as peptide nucleic acids (PNAs), which block transcriptional activity via sequence-specific invasion of double-stranded DNA may be an effective strategy to target cells harbouring proviral HIV DNA. Here we show that a PNA oligomer (PNAHIV), 15 bases in length, linked to a nuclear localization signal (NLS), substantially suppressed HIV-1 replication in chronically infected lymphocytes and macrophages and efficiently prevented mitogen-induced HIV-1 reactivation in lymphocytes, as determined by HIV-p24 antigen production in supernatants and FACS analysis for intracellular HIV accumulation. In contrast, a mismatched PNA did not show any effect on HIV expression. Semi-quantitative RT-PCR and quantitative real-time RT-PCR demonstrated a decrease of HIV RNA expression in infected cells treated by PNAHIV indicating that inhibition of HIV-1 replication occurred at the transcription step. In conclusion, the use of anti-gene PNA to target the HIV-1 proviral DNA in the quest for new antiretroviral agents appears quite promising.

Simard, C., F. Nadon, et al. (1995). "Evidence that the amino acid region 124-203 of glycoprotein G from the respiratory syncytial virus (RSV) constitutes a major part of the polypeptide domain that is involved in the protection against RSV infection." *Antiviral Research* 28(4): 303.

http://www.sciencedirect.com/science/article/B6T2H-3YGV2K1-3/2/575785ce3be67f567ec32f68b02ed432

The first 230 residues of the 298-amino acid glycoprotein G of respiratory syncytial virus (RSV) are sufficient to confer complete resistance to challenge with live RSV, whereas the first 180 residues completely failed (Olmsted et al. (1989) J. Virol. 63, 411-420). The characterization of a protective epitope corresponding to the amino acid region 174-187 of the G protein (Trudel et al. (1991) Virology 185, 749-757) suggests that interruption of this region in the 180 residue truncated polypeptide may be responsible for its inability to confer protection and consequently
that the 174-187 region may play a major role in the protection effected by the protein G. To support these hypotheses, we examined the ability of the amino acid region 124-203 of glycoprotein G to confer protection. The corresponding peptide was expressed as a non-fusion protein in a recombinant vaccinia virus designated VG27. Immunization of BALB/c mice with this recombinant efficiently induced the production of antibodies capable of recognizing both the parental glycoprotein G and peptide 174-187. Furthermore, upon challenge with RSV, a significant decrease of infectious particles was found in the lungs of mice immunized with VG27 as compared with non-immunized mice. Our results suggest that the 124-203 amino acid region of the RSV G protein constitutes a major part of the domain involved in protection.


http://www.sciencedirect.com/science/article/B6T2H-4B22RY0-2/2/490c103f6bffe03ef19e63b34c388a8

It has been known that, in some cases, accumulation of specific mutations in HIV-1 protease leads to multi-protease inhibitor (PI) resistance. We examined the persistence of mutations detected in HIV-1 clinical isolates cross-resistant to the current PIs using an HIV-1 protease restricted library (HXB2 protease in an HIV-1NL4-3 background) in the absence of protease inhibitors. The virus library contained combinations of 0-11 amino acid substitutions (4,096 possible combinations) in the protease-encoding region. We examined the frequency of each amino acid substitution in the library using a T cell line, MT-2. The frequency of the amino acid substitutions V82T/I and L90M decreased rapidly with a short half life (t1/2=34.2, 28.1 and 30.6 days, respectively. Other amino acid substitutions, i.e., L10I, I54V, L63P, A71V and V82A, were well retained (t1/2>36 days). By contrast, the half lives (t1/2) of the D30N and N88D mutations associated with nelfinavir (NFV) resistance were only 7.2 and 1.8 days, respectively. These results indicate that this type of the HIV-1 protease restricted library is useful to evaluate the persistence of PI resistance-associated mutations in the absence of drug selective pressure.


http://www.sciencedirect.com/science/article/B6T2H-3Y6Y3VD-3/2/01a80c66bd2f081a6fb3bce3858cb8b

3'-Azido-3'-deoxythymidine (AZT) treatment in HIV-infected patients is limited by bone marrow suppression including neutropenia and anemia. Previous studies had shown a direct effect of high concentrations of this drug on globin gene expression in K-562 erythroleukemia cells. To better define the mechanism(s) of AZT-induced bone marrow toxicity, the present study evaluates these effects in more relevant human erythroid progenitor liquid cultures, because AZT is 100 times more toxic to human bone marrow cells than K-562 cells. At a clinically relevant concentration of 1 [mu]M, AZT inhibited specifically erythroid cell growth by ~58% as compared with untreated cells. The percentage of cells synthesizing hemoglobin was decreased also by 47% in AZT-treated cells with [beta]-globin mRNA levels accounting for 0.27 pmol in treated cells as compared with 1.44 under control conditions while [beta]-actin levels remained unchanged. Under the same conditions, AZT inhibited the [beta]-globin chain synthesis by ~60% as compared with the control. Consistent with the data described above was the finding that a concentration as low as 0.1 [mu]M of AZT decreased by almost 40% the binding level of the erythroid-specific transcription factor GATA-1. These findings demonstrate that AZT, at clinical relevant concentrations, specifically inhibits [beta]-globin gene expression in human erythroid progenitor liquid cell culture.

We tried to establish whether MT-4 cells that were infected with HIV-1(HTLV-IIIB) at a high multiplicity of infection (m.o.i.=1), and subsequently treated with high concentrations of anti-HIV drugs for several days, would be able to resume virus production after the antivirals are washed away. The HIV inhibitors studied were the nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine and lamivudine, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine and loviride, the acyclic nucleoside phosphonate RT inhibitor (R)-9-(2-phosphonylmethoxypropyl)adenine (tenofovir) and the protease inhibitors (PIs) saquinavir, indinavir and ritonavir. The compounds, at 50 and 500 times their 50% inhibitory concentration (IC50, determined at a m.o.i.=0.01), were added immediately after virus adsorption and removed after an incubation period of 0 (wash control), 24, 48 or 72 h. Virus breakthrough was monitored by microscopical examination of cytopathicity, viral infectivity (yield) and p24 levels in the supernatant. The presence of HIV-1(HTLV-IIIB) proviral DNA was determined after a 72-h incubation period. None of the antiviral drugs studied was able to prevent resumption of viral growth after removal of the compound. Tenofovir, lamivudine and the NNRTIs nevirapine, delavirdine and loviride, at 500 times their respective IC50, were able to delay viral breakthrough for approximately 2-3 days. The NRTI zidovudine and the PIs saquinavir, indinavir and ritonavir, under the same conditions, were not able to delay viral breakthrough at all. Virus recovered upon treatment proved as sensitive to the anti-HIV drugs as wild-type virus. Our results suggest that viral replication at the cellular level was not completely inhibited by drug monotherapy. Consequently, virus rebounded when drug therapy stopped. In conclusion, our findings suggest that drug holidays would result in viral breakthrough, even after virus replication has been previously suppressed by adequate drug levels.
Mycophenolic acid [the active metabolite of the immunosuppressive agent mycophenolate mofetil (MMF)] and ribavirin were found to potentiate the anti-HBV activity of the guanine-based nucleoside analogues penciclovir (PCV), lobucavir (LBV) and 3'-fluorodideoxyguanosine (FLG) and diaminopurine dioxolane (DAPD). Ribavirin and mycophenolic acid are both inhibitors of inosine 5'-monophosphate dehydrogenase and cause a depletion of intracellular dGTP levels. It may be assumed that the 5'-triphosphorylated derivatives of the guanine-based nucleoside analogues, in the presence of reduced levels of dGTP, inhibit more efficiently the priming reaction as well as the reverse transcription and DNA-dependent DNA polymerase activity of the HBV polymerase. This assumption is corroborated by the observation that exogenously added guanosine reversed the potentiating effect of ribavirin and mycophenolic acid on the anti-HBV activity of the guanosine analogues. Our observations may have implications for those (liver) transplant recipients that receive MMF as (part of their) immunosuppressive regimen and that, because of de novo or persistent infection with HBV, need specific anti-HBV therapy.

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To evaluate PCR-generated artifacts (i.e., chimeras, mutations, and heteroduplexes) with the 16S ribosomal DNA (rDNA)-based cloning approach, a model community of four species was constructed from alpha, beta, and gamma subdivisions of the division Proteobacteria as well as gram-positive bacterium, all of which could be distinguished by HhaI restriction digestion patterns. The overall PCR artifacts were significantly different among the three Taq DNA polymerases examined: 20% for Z-Taq, with the highest processivity; 15% for LA-Taq, with the highest fidelity and intermediate processivity; and 7% for the conventionally used DNA polymerase, AmpliTaq. In contrast to the theoretical prediction, the frequency of chimeras for both Z-Taq (8.7%) and LA-Taq (6.2%) was higher than that for AmpliTaq (2.5%). The frequencies of chimeras and of heteroduplexes for Z-Taq were almost three times higher than those of AmpliTaq. The total PCR artifacts increased as PCR cycles and template concentrations increased and decreased as elongation time increased. Generally the frequency of chimeras was lower than that of mutations but higher than that of heteroduplexes. The total PCR artifacts as well as the frequency of heteroduplexes increased as the species diversity increased. PCR artifacts were significantly reduced by using AmpliTaq and fewer PCR cycles (fewer than 20 cycles), and the heteroduplexes could be effectively removed from PCR products prior to cloning by polyacrylamide gel purification or T7 endonuclease I digestion. Based upon these results, an optimal approach is proposed to minimize PCR artifacts in 16S rDNA-based microbial community studies.

http://www.sciencedirect.com/science/article/B6T4B-42M1F7S-3/2/26b0f0ef82018b25d662cb771b5386f1

Microbial community structure in soil sampled from sites contaminated with different levels of heavy metals was assessed by PCR-DGGE analysis of 16S rDNA fragments and MIDI-FAME profiling of total cell fatty acids. Total community DNA was extracted from these soils by three methods to compare their usefulness for generation of representative pools of bacterial community 16S rRNA genes. Crude DNA extracts were purified and then amplified using eubacterial primers. PCR products were analysed by DGGE to obtain bacterial community patterns. Culturable fractions of fast growing bacteria separated from soil colloids by blending and differential centrifugation were also analysed by profiling of cellular fatty acids. PCR-DGGE analysis showed significant differences in microbial community structure between the soils studied, which were related to the contamination levels. Polluted soils could be characterised by a community differing in 'richness' and structure of dominating bacterial populations from those of a pristine soil. The differences in the bacterial community structure were still visible after 10-fold dilution of the target DNA, indicating that even less dominant populations were affected by heavy metals. However, organic matter content, soil type and crop cultivation could also affect the bacterial populations that established in these soils. The direct methods for DNA extraction from soil generated information about the microbial community composition different from that of the indirect method. The latter method was less efficient than both direct methods with respect to the generation of representative pools of bacterial community 16S rRNA genes. The structure of the culturable bacterial community was not dependent on the concentrations of heavy metals in soil, as determined by MIDI-FAME profiling. It is possible that this fraction of soil bacteria was less diverse (dominated by gram-positive bacteria) than the total community analysed at the DNA level without prior cultivation.


http://www.sciencedirect.com/science/article/B6T4B-4BWYHR4-2/2/bdf9c63a3996256803e3e9d6d99b7329

Thirty rhizobial isolates that nodulate common bean (Phaseolus vulgaris L.) plants were obtained from a range of cultivated soils covering 16 geographical sites in Jordan. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for nodA, nifH and glnII genes and sequencing methods showed that the rhizobial isolates formed two main groups. The first, which makes up 80% of isolates, were identified as Rhizobium etli while the second group comprising the remaining 20% were related to Rhizobium tropici. These findings indicate that R. etli is the predominant rhizobium that nodulates common bean in Jordan.

This study aimed to evaluate the amino acid-chelated trace elements as dietary supplement to rainbow trout. Three diets were formulated containing trace elements either from the inorganic salt (SF) or amino acid-chelate (AM). Diets 1 (SF) and 2 (AM) contained the same amount of trace elements from inorganic and amino acid-chelates, respectively. Diet 3 (AM-Hf) was added with trace elements from amino acid-chelated at one-half of their levels in Diets 1 and 2. Each diet was fed for 15 weeks to three groups of 30 fish each, with an average weight of 1.52±0.21 g. Growth of fish was not affected by the treatment (P>0.05). However, bone (PPPPPP<0.05) than the inorganic salt. Half supplementation of those fed the elements from AM was at par with the full provision from the inorganic source tested.


Amplification by the polymerase chain reaction (PCR) was done to determine the presence of red sea bream iridovirus (RSIV) in sea perch (Lateolabrax sp.) imported from China, targeting four genomic regions, the ribonucleotide reductase small subunit (RNRS) gene, the adenosine triphosphatase (ATPase) gene, the DNA polymerase (DPOL) gene, and the Pst I restriction fragment, which have been considered to be potential target regions for the diagnosis of RSIV infection. In contrast to two other RSIVs, RSIV Sachun and RSIV Namhae, which were isolated in Korea, the newly isolated RSIV CH-1 was not detected by PCR with one reported primer set specific for the Pst I restriction fragment. We cloned full-length Pst I restriction fragments from the genomic DNA of three different RSIVs after PCR with primers derived from regions just outside the Pst I restriction fragment using previously reported sequences (4436 bp long and designated as the K1 region), and sequenced the resulting cloned DNA. Two locations of sequence variation, around positions 24-41 and 425-446 in the Pst I restriction fragment, were found in closely related viruses. Nucleotide differences at the first position in RSIV CH-1 prevented the binding of the sense primer derived from the sequence of the reference strain (RSIV Ehime-1) and appeared to cause a negative result in PCR amplification of the targeted Pst I restriction fragment. For differentiation of these three different RSIVs, two primers, NF and CR, specific to RSIV Namhae and CH-1, respectively, were strategically designed by taking advantage of the nucleotide substitutions and a deletion of three successive nucleotides in the two variable positions of the Pst I restriction fragment. The specificity of these primers in general PCR was confirmed by using viral genomic DNAs and plasmids containing target genes of the different RSIVs as templates. In multiplex PCR with all four primers derived from two variable and two conserved positions in the Pst I restriction fragment of RSIV, it was possible to distinguish the three different RSIVs, RSIV Namhae, Sachun, and CH-1, from one another depending upon the different sizes of the PCR amplicons. Thus, the multiplex PCR developed in this study using a minimum number of strategically designed primers provides a basis for rapid and simple differentiation of RSIVs from different hosts or countries merely by the observation of the predicted amplicons and without the necessity of making nucleotide sequence comparisons.

http://www.sciencedirect.com/science/article/B6T4D-40YYFJB-N/2/28830942d86b900823b943b4eede8c96

Determination of the onset of amylase production in marine fish larvae is difficult due to their small size and the possible presence of exogenous amylases from prey organisms in the diet or from the gut flora. In order to develop a sensitive PCR-based assay for the detection of fish-specific amylase in larvae, a complete cDNA and partial genomic sequence, the first reported from a teleost fish, were determined from winter flounder. The complete cDNA for alpha amylase is 1539 bp and the deduced polypeptide sequence is 512 amino acids, including a putative 15 amino acid signal peptide. The molecular weight of the mature protein is 55,769 Da and the predicted isoelectric point is 6.76. Southern hybridisation analysis showed that the winter flounder amylase cDNA could be used to detect homologs in other species, particularly flatfish, and that there are likely two copies of the gene in the winter flounder genome. The winter flounder genomic sequence corresponding to amino acids 194-404 (including three introns) was amplified by the polymerase chain reaction (PCR) and the sequence used to design primers for PCR-based assays for amylase gene expression in larval and adult fish. The levels of expression of the amylase gene from larvae sampled at 5, 13, 20, 27 and 41 days post-hatch (dph) were determined using the housekeeping gene, actin, as a control. Amylase transcripts were first detected at 5 dph, peaked at 20 dph and then decreased during metamorphosis. The amylase gene is highly expressed in adult winter flounder. This sensitive assay will be useful for investigating amylase gene expression under different feeding conditions and help in the development of optimal diets.


There is currently concern and controversy in the shrimp industries of the Americas about the risk posed by the importation and reprocessing of shrimp infected with white spot syndrome virus (WSSV) and yellow head virus (YHV). To further understand the risk, more knowledge concerning the quantitative virus load of infected shrimp is needed. The present study was carried out to better define, using qualitative and quantitative methods, the relative virus load of shrimp heads and tails. For these studies, specific pathogen-free (SPF) Penaeus vannamei were infected with WSSV. Emergency harvest of these shrimp was simulated by collecting the infected shrimp at the onset of postinfection mortalities and determining the relative virus loads of the head and tails by quantitative real-time PCR and histology methods. Routine histology and in situ hybridization assay with a WSSV-specific DNA probe demonstrated qualitatively similar levels of WSSV infection in the heads and tails of experimental infected shrimp. The novel real-time PCR method demonstrated quantitatively that the head had a slightly higher WSSV load than did the tail. However, since the tail represents 58% of the total body weight, the total virus load on a per weight basis turns out to be similar in the head (49%) and tail (51%) of the same shrimp with acute phase WSSV infections. In proportion to the total tail weight, the virus load of the peeled shell represents 55% of the total viral load in tail.


Means to control infection by pathogenic organisms are needed to help ensure that aquaculture is not a source for the spread of infectious diseases in wild and cultured stocks. Questions often arise as to whether larval or juvenile stages become infected in the hatchery or nursery phase of production, and if so, how they might be protected. To help answer these questions, we utilized both traditional and molecular diagnostic methods to detect two eastern oyster, Crassostrea virginica, pathogens (Haplosporidium nelsoni, cause of MSX disease and Perkinsus marinus, cause of Dermo disease) in larval and juvenile oysters reared at a hatchery/on-shore nursery receiving water in which both parasites are enzootic. Our study indicated that filtration of water to 1 μm using a cartridge filter followed by exposure to 30,000 μW s-1 cm-2 ultraviolet (UV) irradiation was an effective means of preventing infections of the larvae and post-set. Once the juveniles were moved from the highly treated hatchery water to an upweller nursery receiving only roughly (150 μm) bag-filtered water, however, they became infected by both parasites.


http://www.sciencedirect.com/science/article/B6T4D-49M6RDP-5/2/07d168ddc176899b33c3ee9b5b5230a2

Partial or total replacement of fish meal by a mixture of plant protein sources (corn gluten meal, wheat gluten, extruded peas, rapeseed meal) balanced with indispensable amino acids (IAA) was examined in juvenile gilthead sea bream (Sparus aurata) over the course of a 12-week growth trial. A diet with fish meal (FM) as the sole protein source was compared to diets with 50%, 75% and 100% of replacement (PP50, PP75, PP100). Protein retention was improved with more plant protein supply, and just a slight decrease in the final weight gain was found in fish fed PP50 and PP75 diets. However, in the PP100 group, weight gain was depressed up to 30% mainly as the result of a marked reduction of feed intake. These fish also showed a lower fat gain along with a marked hypocholesterolemic effect. Dietary treatment did not alter the hepatic activity of amino acid catabolising enzymes (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glutamate dehydrogenase (GDH)), although the size of the total muscle free amino acid (FAA) pool was increased by more plant protein supply. The activity of the somatotropic axis also varied among experimental groups, and the up-regulation of circulating growth hormone (GH) levels with a high plant protein supply followed the decrease in growth rates, plasma levels of insulin-like growth factor-I (IGF-I), and liver mRNA transcripts of IGF-I and GH receptors. This catabolic feature evidenced a liver desensitisation to the anabolic action of GH in the PP100 group, and to a lesser extent in the PP75 group. Taken together all these findings, up to 50-75% of fish meal replacement seems to be feasible with IAA supplementation, but further research is needed to fully identify the responsible factors for the depressed feed intake in order to achieve a full replacement in a fish species having high dietary protein requirements.

Parentage of offspring in a stock of a cultured Japanese flounder Paralichthys olivaceus was determined using four hyper-variable microsatellite DNA loci with many unique alleles. It was found that only 57% of the 14 broodstock parents actually contributed to the production of offspring in this experiment. The number of alleles per locus in the offspring was reduced 29% compared to the broodstock. The average heterozygosity of offspring (He=0.883) was significantly lower (P<0.01) than that of their parents (He=0.943). In this study, use of hyper-variable microsatellite markers with many rare alleles was effective for unambiguous parentage determination and estimation of genetic diversity in hatchery and natural populations of Japanese flounder. And it was shown using parentage determination that the difference between the number of parents contributing to reproduction and the number of fish stocked caused loss of genetic variability.


Arbitrary primers and the DNA polymerase chain reaction (PCR) technique were applied to study genetic variation between different strains of the crayfish plague fungus, Aphanomyces astaci. Eight different primers among 15 tested were chosen for a comparative analysis of the different A. astaci strains. On average, each primer gave rise to 5-8 bands and a majority of the bands was polymorphic for at least some strains. Two main groups among the different Swedish isolates were clearly discernible. One group included isolates from the indigenous crayfish species Astacus astacus and one isolate from As. leptodactylus originating from Turkey. The other main group included fungal isolates from both As. astacus and the introduced North American crayfish Pacifastacus leniusculus. In this latter case it seems likely that the introduced American signal crayfish has served as a vector and transmitted the pathogen to the indigenous crayfish.


The nucleotide sequences of DNA fragments amplified by polymerase chain reaction (PCR) from four different genomic regions of nine red sea bream iridoviruses (RSIVs) isolated from different species of fish, different areas and in different years in Korea were compared with the reported reference sequences. One isolate, RSIV Namhae, showed 100% homology to the reference sequences, while the other eight isolates, which appeared to contain identical nucleotide sequences, showed 96.6-98.9% homology with reference sequences depending upon the target regions of PCR gene amplification. However, differences in nucleotide sequences were not apparent between the RSIVs isolated in different locations, in different years or in different host species. We also cloned and sequenced the 3' end flanking region (K1) of the DNA polymerase (DPOL) gene using the cassette ligation-mediated PCR method. This sequence was 4436-bp long and possessed two open reading frames (ORF-1 and ORF-2) oriented in opposite directions. The putative proteins encoded by these two ORFs could not be characterized by comparison with the proteins of other species in the data banks. The presence of the ribonucleotide reductase
small subunit (RNRS) gene at the 3′ end of the K1 region allowed us to determine that these two genes, RNRS and DPOL, are separated 5508 bp and oriented in the same direction in the genome of RSIV. Moreover, it is of interest that a PstI-restriction fragment, of which the sequence but not the location within the RSIV genome had previously been reported, is located at nucleotide positions from 1096 to 2054, extending from within the ORF-1 region, spanning the intervening sequence between ORF-1 and ORF-2, and extending into the ORF-2 region. Various repeating sequences up to 86 bp were present at the 3′ ends of ORFs, especially within the nucleotide sequences at the 3′ terminus of ORF-2. No similarities were detected when the DNA sequences of the K1 region were compared to the DNA sequences of a repetitive element in the genome of other iridoviruses.


http://www.sciencedirect.com/science/article/B6T4D-4D98XYS-4/2/a2d78e0f24a06d3a4597fb0e7c7b6d13

To determine and discriminate the types of tetracycline (Tc)-resistance determinants (tet), we developed a multiplex polymerase chain reaction (PCR). With minimized numbers of primers derived from the variable and conserved regions of six different types of tet genes, tet(A)-(E) and (G), the multiplex PCR was sensitive and specific enough to discriminate the various types of tet genes, even multiple tet genes in an individual resistant isolate, by the different sizes of the resulting PCR products. Each of 20 Tc-resistant Edwardsiella tarda (Ed. tarda) isolates from diseased fish from aquaculture farms in Korea carrying either one or two tet genes of types (A), (D), (B), or (G) gave PCR products of the appropriate lengths. Among the four types of tet genes found in Ed. tarda, two types, tet(A) and (D), were always present on mobile plasmids, and the other two types, tet(B) and (G), were located on the nonmobile nucleic acids. This is the first time that either of these two genes, type (B) or (G), have been found in Ed. tarda isolates. The two most common types of Tc-resistance determinant were, tet(A) and (D), occurring in 55% and 45% of total Tc-resistant Ed. tarda isolates, respectively.


http://www.sciencedirect.com/science/article/B6T4D-41XV7CM-1/2/58cd3871252c03f0384fc2bd5ad4dc9

A PCR method for the detection and differentiation of Vibrio vulnificus strains was developed as an alternative to culture methods by using combined primers directed against the variable regions of 16S rRNA. Primers designed from two variable regions of the Vibrionaceae 16S rRNA (corresponding to nucleotide numbers 1006 to 1023 and 1278 to 1258 in Escherichia coli 16S rRNA) was found to be species-specific for V. vulnificus by PCR. Additionally, tri-primer PCR of 16S rRNA was evaluated for the differentiation of V. vulnificus strains. Although the third primer, which was derived from the variable region, positions 454 to 473, cannot discriminate V. vulnificus from other bacteria, it was used to avoid the detection of type B 16S rRNA of this organism in PCR. The resulting 825 bp fragment in the presence of the 273 bp fragment, which is specific to V. vulnificus, in tri-primer PCR clearly differentiated type A 16S rRNA strains from type B. Enumeration of V. vulnificus in the samples of oyster and environmental samples was done by most probable numbers' (MPN) method of five preenrichment tubes of alkaline peptone water supplemented with polymyxin B following up the confirmation of positive tubes by streaking the samples onto mCPC agar or by 16S rRNA gene amplification. Higher numbers of presumptive V.
vulnificus confirmed by selective media compared with those confirmed by PCR method in MPN method suggested that there would be some bacteria that cannot be discriminated from V. vulnificus on mCPC agar in environmental samples. In the biotyping of the V. vulnificus isolates in oyster samples, the majority of the strains (92.5%) belonged to biotype 1, and 7.5% of the strains belonged to biotype 2. However, strains of 16S rRNA of V. vulnificus isolates in the marine environment determined by tri-primer PCR appeared to be 35% type A and 65% type B. These results implied that the marine environment can serve as reservoir of both V. vulnificus biotypes 1 and 2, and strains of 16S rRNA type B were more frequent than strains of type A in that environment.


http://www.sciencedirect.com/science/article/B6T4D-4BMTJ11-6/2/0c4c007f65527bd41383b2ec02890651

In order to assess the utility of microsatellite DNA markers for detecting changes of genetic diversity in hatchery strains and for estimating their genetic relationships, we used six microsatellite markers to estimate the level of genetic diversity within three hatchery strains and two wild populations of Pacific abalone, and compared the degree of genetic differentiation between them. High polymorphism at the microsatellite loci was found within both hatchery and wild abalone populations. Compared to wild populations, all the hatchery strains showed less genetic variation as revealed in lower number of alleles and lower expected heterozygosity, indicating that bottleneck effects occurred when each strain was founded. Significant differentiation was found between the hatchery strains, and between the hatchery strains and wild populations (Fst range: 0.059-0.427; Rst range: 0.056-0.351), and no obvious difference was detected between the wild populations (Fst=0.004; Rst=0.007). According to the neighbor-joining tree topology constructed on the basis of genetic distances among individuals, almost all individuals from each hatchery strain were closely clustered, demonstrating the feasibility of microsatellite analysis for discrimination between hatchery strains. The results obtained in this study indicate that it is necessary to genetically characterize the abalone strains that are being released every year in order to monitor the effect on the genetic diversity of wild populations.


http://www.sciencedirect.com/science/article/B6T4D-3S12526-J/2/b71ad64c7f20b0ace61e575654c64fce

Two isolates of Aphanomyces astaci obtained from diseased white-clawed crayfish (Austropotamobius pallipes) in Herefordshire, England were compared against representative isolates of three groups of the fungus found in Sweden and one from Spain by means of random amplification of polymorphic DNA (RAPD). The English isolates proved to be very similar to a Swedish strain which is considered to have been introduced from North America with shipments of the signal crayfish (Pacifastacus leniusculus) from 1970 onwards, and has since spread to indigenous populations of noble crayfish (Astacus astacus). This strain has not been found to be involved in recent incidences of crayfish plague in Turkey and Spain. It is therefore most likely that at least some of the outbreaks of crayfish plague in England resulted from imports of P. leniusculus from northern Europe after 1970, or directly from North America.
Transmission of exotic pathogens occurs through a variety of means, including migration with humans and animals, rapid transit by land, sea or air or through the shipment of infected frozen food products. White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) have caused mass mortalities of cultured shrimp in Asia beginning in 1992. In 1995, these viruses appeared for the first time in the Western Hemisphere causing high mortalities in farm reared shrimp in Texas, USA. The purpose of this study was to determine if WSSV and YHV are present in frozen shrimp products imported into the United States from Asia. Infectivity assays, transmission electron microscopy (TEM), and polymerase chain reaction (PCR) showed these viruses were detectable and infectious in frozen shrimp imports. Frozen shrimp were used to infect indicator shrimp (Penaeus stylirostris) which resulted in mortalities. The cause of these mortalities was determined by histology and TEM to be by YHV. PCR confirmed the presence of WSSV in the frozen, purchased products. The results from this study indicate that exotic shrimp pathogens can be transmitted via imported frozen products.

This study examined the viral copy number as determined by real-time RT-PCR, in different tissue samples from Penaeus vannamei exposed to Taura syndrome virus (TSV). Two routes of exposure, injection and per os, were investigated. Six different body parts from each shrimp were assessed for viral copy numbers. Eight shrimp were analyzed per treatment. In addition, eight specific pathogen free (SPF) P. vannamei were analyzed and served as a negative control. The tissue samples examined included: whole tail muscle, tail muscle (shell removed), gills, pleopods, head (cephalo thorax with the hepatopancreas included) and tail fan. The results from these experiments showed a significant level of difference between the SPF and the injection treatments. As was expected, there was also a significant difference between the negative control and the per os treatment groups. There was no significant difference between the viral copy number contained in different body parts from the injection experiment. These results contrasted to the per os results, in which there was a statistically significant difference between tail/gills, tail/head, tail/tail fan, whole tail/tail fan and pleopods/tail fan. The tail samples had the lower viral copy numbers, as did the whole tail and pleopods when compared to the tail fan. The mean viral copy number per nanogram of total RNA (x cn/ng tRNA) extracted in the injection study ranged from 1.4 x 105 in the gills to 2.3 x 105 in the whole tail. In the per os experiment, the x cn/ng of extracted tRNA ranged from 2.5 x 104 in the tail muscle to 4.3 x 105 in the head. When these values are converted to mean viral copy number per gram (x cn/g) of tissue, the values increased in range. In the injection study, the x cn/g of tissue ranged from 1.2 x 109 in the tail fan to 7.4 x 109 in the head. These values contrast to the x cn/g of tissue in the per os study in that the lowest value of 1.7 x 108 was in the tail muscle and the highest x, 1.7 x 1010, was in the head. Overall, all body parts from infected shrimp, regardless of treatment type, quantitatively analyzed by real-time RT-PCR, determined the presence of TSV.
The domestication of rainbow trout, *Oncorhynchus mykiss*, has led to the development of distinguishable isolated populations. In this study, five different strains of rainbow trout from the Northwest, USA were examined for variability in growth, immunological response, and genetic diversity. Growth rates for the different strains were monitored and compared for 28 weeks, with the animals fed at a fixed rate or to apparent satiation. Feed conversion ratios (FCR), specific growth rates (SGR), and thermal-unit growth coefficients (TGC) for the entire period were calculated for each of the strains. The different strains were also evaluated immunologically with infectious hematopoietic necrosis virus (IHNV), and their post-immunization antibody neutralization titers were monitored for a period of 12 weeks. Using microsatellites, the genetic variability between the strains was examined from a representational sample of the population of each strain. Fastest growing strains grew to a set weight of 350 g more rapidly regardless of whether they were fed at a fixed rate or to apparent satiation. These faster growing strains also exhibited a lower FCR and higher SGR and TGC values, and higher percentage protein retention. The IHNV neutralization titers for the strains varied considerably with one high humoral response group and one low humoral response group. The remaining three strains clustered approximately midway between the other two. Genetically, the strains exhibited a pattern of wide divergence, with only 9 common alleles out of a total of 89 different alleles between the five strains. As expected, commercial aquaculture strains reared locally were genetically more similar, and strains that have undergone intense selection tended to have a strong correlation between reduced genetic variability, FCR, and SGR as compared to noncommercial strains.


Sequence variation was detected by polymerase chain reaction (PCR) and direct sequencing of a 295-nucleotide region of the mitochondrial cytochrome b gene of Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) sampled around Iceland. Four haplotypes were found within Atlantic salmon, no variation was found within Arctic charr or brown trout. Synonymous substitution rates among the species were according to a molecular clock. There was a significant transition/transversion bias but apparent transitional bias among purines and pyrimidines was not significant, given permissible mutations which would not alter the amino acid sequence. The amino acid sequences were identical among the species where intraspecific variation did not exist.


The reproductive readiness of the marine shrimp *Litopenaeus vannamei* (formerly *Penaeus vannamei*) cultured in brackish water was characterized by applying morphological, physiological, and molecular tools. The shrimp were cultured on two commercial shrimp farms in brackish water.
that was pumped from artesian wells that tap into a geothermal aquifer. The shrimp populations exhibited a bimodal growth curve with the females being significantly bigger than the males at the end of the growout period. Some male shrimp started to develop spermatophores about 6 months after the first post-larval (PL0) stage, and some with developed, normal-looking, white spermatophores were observed 8 months after PL0. The sperm count in these males was 10.1 x 106±5.8 x 106 cells per compound spermatophore, and 81.6±19.8% of the cells were spiked. Melanization of the males, which eventually affected about a third of the male population, first became evident before the appearance of white spermatophores. Female ovaries were transparent and appeared to be arrested in a previtellogenic stage. However, beyond a weight/age threshold of 20 g/8 months, some of the ovaries had become opaque and the vitellogenin gene was found to be expressed in the ovary—but not in the hepatopancreas—of 7 out of 10 females. A unique case of a 46.8 g female with a fully developed ovary that was found in the brackish-water-held broodstock is reported.


http://www.sciencedirect.com/science/article/B6T4D-4C52NYK-2/2/f9770452e6f904b344e20605e548b8e7

In the present work, we report the characterization of the partial cDNA sequence of a bile salt-activated lipase (BAL) cDNA from haddock. The predicted polypeptide encoded by the cDNA sequence contains the bile salt-binding site characteristic of all BALs at amino acid positions 36-45, and the lipid-binding site thus far only reported in fish BALs starting at position 345. Other features of BAL are also present including: the active site serine motif at positions 111-117, the catalytic triad formed by the residues S114, D239 and H358, and an N-glycosylation site at position 107. The relative levels of BAL gene expression were determined in haddock larvae during ontogeny by reverse transcription-polymerase chain reaction (RT-PCR) with the earliest detectable transcript levels identified at hatch. Using in situ hybridization, the BAL transcripts were localized consistently in the pancreas of haddock larvae from mouth opening until 401 degree days (DD). Using biochemical techniques, the specific activity of BAL was found to decline significantly over time. Our results also suggest that haddock larvae are capable of digesting lipids at the time of mouth opening.


http://www.sciencedirect.com/science/article/B6T4D-3W2XKYC-G/2/a533a428639946a21f0dea50846ab32a

The effect of simultaneously cultivating the pearl oyster Pinctada martensi and the red alga Kappaphycus alvarezii on growth rates of both species was investigated in laboratory and field studies conducted from December 1993 to June 1995. The two study sites were in subtidal areas 100 km apart off the east coast of Hainan Island, China. Pearl oysters were cultivated in the center of an algal farm and red alga was cultivated in the center of the pearl oyster farm. These field experiments showed higher growth rates of both P. martensi and K. alvarezii in a co-culture system than in a monospecies culture system. Laboratory studies showed that the algae removed nitrogenous wastes released by pearl oysters. Algae treated with pearl oyster wastes grew much faster than those without oyster wastes. Algae treated with the seawater to which NH4Cl, NaNO3 and NaNO2 were added grew at the same rate as those treated with natural seawater containing oyster nitrogenous wastes, suggesting that enhanced growth of algae in the co-culture system was largely due to nitrogenous metabolites of the pearl oysters. In the co-culture, growth of pearl
oysters was positively influenced by the presence of rapidly growing algae but when seawater temperature decreased below 20 [°C], the algae grew slowly and there was no measurable benefit of mixed culture to either algae or pearl oyster.


http://www.sciencedirect.com/science/article/B6T4D-3Y51T0X-3/2/790aa0c84f5b63a3c6f5478f1f3173c7

Experiments on ingestion rates, colonization and impact of inhibitory producer substances bacteria (IPB) on larvae of Argopecten purpuratus, were carried out to evaluate potential use of bacteria as probiotics in cultures of this Chilean scallop. Three selected strains, named as 11, C33 and 77, obtained from larval cultures of A. purpuratus were tested at different concentrations and incubation times. After 6 h of incubation at a concentration of 106 cells ml-1, A. purpuratus larvae ingested cells of strains 11 and 77, but not those of C33. When comparing bacterial incorporation among these strains, the 77 became the dominant bacteria of the larval microflora, causing no differences in larval survival at different bacterial concentrations. Our results suggest that strain 77 appears as a potential probiotic for scallop larvae and hence, as a promising method to control and prevent infections in hatcheries systems.


http://www.sciencedirect.com/science/article/B6T4D-44TV9D6-1/2/aacd283ed0d7ebc97a37428a81c65f3c

Genetic divergence within and between hatchery strains and wild populations of Japanese flounder Paralichthys olivaceus was assessed by means of microsatellite and mitochondrial DNA (mtDNA) sequencing analysis. All of the 11 microsatellite loci screened in this study showed marked polymorphisms. Sequences of the mtDNA control region of Japanese flounder were also highly variable: of approximately 443 base pairs (bp) sequenced, 132 variable sites comprised of 149 base-substitutions were found among the 490 individuals. Marked reductions of genetic variability in the hatchery strains compared with the wild populations were observed in terms of number of both microsatellite alleles and mtDNA haplotypes, and mtDNA haplotype diversity. Both molecular markers yielded high values of FST ([Φ]ST) between the hatchery strains, and between the hatchery strains and wild populations. We conclude that, based on the reduced genetic variability observed in all the hatchery strains examined, bottleneck effects occurred when each strain was founded.


http://www.sciencedirect.com/science/article/B6T4D-483STD5-1/2/6b4750624727fef716adea015456810a

Effective population size in captive populations is affected by several factors such as the number
of contributing broodstock to the next generation, sex ratio of parents, and variations in family size, which can be accurately evaluated by examination of the pedigree structure in the populations of interest. Here we present an example of microsatellite-based pedigree tracing in a hatchery strain of Japanese flounder Paralichthys olivaceus to be stocked into natural sea areas. We also detail the potential effect of selective operations (size selection) on the pedigree structure. The hatchery strain we screened was founded by using 18 wild captives (6 males and 12 females) through the use of the mesocosm spawning method, and the pedigree of the offspring including 113 individual larvae collected within 24 h after hatching, 216 individuals of 1 month old, and 407 individuals of 4 month old was unambiguously identified. The contribution of candidate broodstock to the next generation was highly skewed as the contribution to almost all of the offspring was monopolized by a single male, and a half of the females did not produce any offspring. The contribution of one family to the released fish selected for larger size (total length) was significantly high, while those of other two families were low (P<0.008).


http://www.sciencedirect.com/science/article/B6T4D-3Y9G6WN-1/2/22607991f79785bd9834f76f8f7b8fd

The culturability of the intestinal microflora of 48 rainbow trout was detected by comparing direct microscopic counts (4',6-diamidino-2-phenylindole, DAPI) with plate counts (tryptone soya agar, TSA). In general, a high percentage (average 50%) of the microflora could be cultured. The counts of the intestinal microflora varied 3-5 log units between fish within the same sampling point. A total of 504 bacteria were identified by physiologic criteria and 153 strains also by partial sequencing of the 16S rRNA gene. High agreement was found between classical and molecular identification. The dominant intestinal microflora was identified as bacteria belonging to the gamma subclass of Proteobacteria (of the genera Citrobacter, Aeromonas and Pseudomonas), to the Gram-positive bacteria with low G+C-content (of the genus Carnobacterium) and as bacteria belonging to the beta subclass of Proteobacteria. However, the composition of the intestinal microflora showed high variation among three investigated fish farms and also at different time points within one fish farm.


http://www.sciencedirect.com/science/article/B6T4D-3RXXXTD-C/2/55b187eb8ad1ba8f2683d3a312f0d9dc

Nuclear DNA was isolated from the blood cells of catfish representing three families (clariidae, pangasiidae and ictaluridae) for analysis by polymerase chain reaction (PCR) and restriction enzymes. Primers specific for the CH4 exon of the gene encoding the immunoglobulin M heavy chain of channel catfish (Ictalurus punctatus) were used. Nuclear DNA amplified with these primers yielded a single band of about 300 base pairs (bp) for Clarias macrocephalus, Pangasius gigas, Pangasius hypophthalmus and the hybrid of P. gigas X P. hypophthalmus. However, the same primers yielded two DNA bands of about 300 and 340 bp in Clarias gariepinus and in the hybrid of C. macrocephalus X C. gariepinus. Nucleotide sequences of the amplified DNA were determined for I. punctatus, C. macrocephalus, P. gigas and P. hypophthalmus. Based on the DNA sequence data, the restriction enzyme Hpal was used to further characterize the PCR products of P. gigas, P. hypophthalmus and their hybrid. Digestion with this restriction enzyme yielded one DNA band (300 bp) for P. gigas, two bands (100 and 200 bp) for P. hypophthalmus.
and three bands (100, 200 and 300 bp) for the hybrid. These findings would aid in identifying genetic contributions in hybrid, androgenetic, gynogenetic and polyploid catfish.


http://www.sciencedirect.com/science/article/B6T4D-3W31BC5-4/2/04cf67b7dba00bc3d921d3c781f6f6e38

We describe a novel random amplified polymorphic DNA (RAPD) based technique to rapidly assess the overall success of treatments designed to induce gynogenesis. To test this technique we produced white sturgeon (Acipenser transmontanus) meiotic gynogens. A total of 108 putative gynogens of known parentage from four different experimental treatments were screened using RAPD primers which were known to generate sire-specific markers. Only two individuals showed amplification of sire-specific markers indicating that they had received some paternal inheritance and were not true gynogens. This simple RAPD-based technique could be generally applicable for the verification of gynogenesis or androgenesis in other species, especially those which lack suitable phenotypic markers to trace the transmission of parental inheritance. We also determined the ploidy of 2469 diploid, triploid, tetraploid and mosaic sturgeon by using a Coulter Counter to analyze erythrocyte nuclei size and verified the results with flow cytometry. The Coulter Counter proved to be a rapid and accurate technique for ploidy analysis in sturgeon.


http://www.sciencedirect.com/science/article/B6T4D-3Y45FPW-5/2/582f01374640c0a3ebd2ed0e8cd77a3b

The culture of striped bass or its hybrids is currently one of the fastest growing segments of aquaculture in the United States. Although this industry is still in the early stages of development, it is already estimated that cultured striped bass and hybrids exceed that of the wild harvest. One major problem limiting the growth of the industry is the dependency on wild brood stock for seed supply. The Crane Aquaculture Facility (CAF) maintains the largest Chesapeake Bay (Maryland, USA) population of captive (F1) and domestic (F2 or greater) striped bass. These striped bass originated from wild populations of Chesapeake Bay where hybrids of Morone exist sympatrically, and where evidence of introgressive hybridization among Morone has occurred. Given this evidence, we felt it was imperative to screen all of the CAF stock for genetic purity before selective breeding efforts were initiated. We utilized genomic DNA techniques to validate genetic purity because of the ease of sampling and the high level of sensitivity to introgressive hybridization. No white bass alleles were found among the samples tested. Thus, white bass alleles if present at all are extremely rare in the CAF striped bass stocks.


http://www.sciencedirect.com/science/article/B6T4D-45TTPPW-5/2/a654e103b28f63f102be3ebd7b50b154
Multidrug-resistant isolates carrying the chloramphenicol resistance gene were obtained from aquatic farms in various locations in Korea and studied to determine the distribution and origin of the cat gene. Out of the 134 isolates examined, 10 showed multidrug resistance to several different antibiotics including chloramphenicol (CP). One of these 10 multidrug-resistant bacteria, Vibrio damsela JE1 (V. damsela JE1) contained a transferable R plasmid encoding CP and tetracycline (TC) resistance genes. The presence of the R plasmid was confirmed by conjugation using the chromocult medium (CC) as a selective and differential medium for transconjugants with identification based on the growth or colors of the colonies. To determine the types of chloramphenicol acetyltransferase genes (cat), polymerase chain reaction (PCR) with primers derived from the variable and conserved regions of different types cat genes, appeared to be a very specific and sensitive method. Additionally, we developed the multiplex PCR that allowed us to determine the types of cat genes by the different sizes of the resulting PCR products in a single reaction tube. With the PCR method, we determined that all multidrug-resistant isolates of Vibrio from the farms of the South and East Sea that contained the cat gene carried type IV gene in an R plasmid or other nucleic acids, and the remaining isolates including Edwardsiella tarda, Aeromonas hydrophila and Shewanella sp. carried the type II gene. This result suggested that the type of cat gene in the multidrug-resistant bacteria originated from a very limited environmental or biological source and was not dependent on the location of the area of isolation in Korea.

Aquatic Botany (2)


http://www.sciencedirect.com/science/article/B6T4F-3S5BM03-B/2/1d808499cc943c3498d505872f18165e

Halophila johnsonii Eiseman is a seagrass that is restricted in distribution to the Indian River of Florida from Sebastian Inlet on the north to Biscayne Bay on the south, and currently is being considered for listing as a rare/endangered species by the National Marine Fisheries Service (NMFS). At the time it was described as a new species in 1980, no staminate flowers had been reported. After numerous searches in the Indian River in the late 1980s and early 1990s and after culture in the laboratory, only pistillate flowers are known. DNA testing indicates that H. johnsonii is distinct from H. decipiens Ostenfeld. Isozymes and sulfated flavonoids had suggested earlier that H. johnsonii may be closely related to the small-leaved plants in H. minor (Zoll.) den Hartog complex that are widely distributed in the Indo-Pacific. H. johnsonii may represent the vegetative development of a single pistillate clone.


http://www.sciencedirect.com/science/article/B6T4F-3S5BM03-3/2/734c7359d67b4c9a5c3ccf72140753cb

The phenetic relationships among forty-four accessions of Hydrilla verticillata from various regions of the world were determined using random amplified polymorphic DNA (RAPD) analysis of bulked genomic samples. Five primers were used producing a total of 85 resolvable,
polymorphic bands. The accessions were compared using Gower and Dice metrics, clustered using unweighted pair-group arithmetic average clustering (UPGMA) and consensus algorithms, and factored using principal coordinate analysis. Four major clusters (Asian, Australian, Indonesian, monoecious U.S.) and one minor outlier cluster (Japan/Poland) were identified. The U.S. dioecious accessions formed a group closest to an accession from Bangalore, India, possibly lending credence to historical reports that it was imported from Sri Lanka. The U.S. monoecious plants cluster with an accession from Seoul, Korea. Accessions from Taiwan, Burundi, and Panama join the Asian cluster late. The New Zealand accessions cluster loosely with those from Australia. The use of band intensity in combination with the Gower similarity coefficient generated a cophenetic correlation coefficient (similarity matrix vs. UPGMA matrix) of $r = 0.92$, superior to that for the corresponding Dice metric ($r = 0.85$).

Aquatic Toxicology  (6)


http://www.sciencedirect.com/science/article/B6T4G-4CXKG2J-2/2/e272df2116afa7a30ef88a26702eabda

Metallothionein (Mt) has been considered as a molecular marker of metal pollution in aquatic ecosystems. Less is known about the expression of mt gene during embryogenesis. Here, we report the cloning, sequencing, and the expression pattern of mt gene during developmental stages in zebrafish. The zebrafish embryogenesis when takes place in a medium containing a dosage of 1000 [mu]M zinc resulted in high mortality, indicating the deleterious effect of zinc on development. The zebrafish mt gene consists of three exons encoding 60 amino acids with 20 conserved cysteine residues. RT-PCR result indicates the maternal contribution of Mt transcripts. Using digoxigenin (DIG)-labeled anti-sense RNA probe, whole-mount in situ hybridization was performed to observe the expression pattern of zebrafish mt gene during embryonic and early larval stages. Stronger as well as ubiquitous expression of mt gene during early embryonic stages narrowed to specific expression after hatching. The mt promoter region contains seven copies of putative metal-responsive elements (MREs), which are shown to be important for the high level activity by deletion analysis. The expression of mt gene during embryogenesis implies its significant role on development.

Hestermann, E. V., J. J. Stegeman, et al. (2002). "Relationships among the cell cycle, cell proliferation, and aryl hydrocarbon receptor expression in PLHC-1 cells." Aquatic Toxicology 58(3-4): 201.

http://www.sciencedirect.com/science/article/B6T4G-45S3JM5-7/2/8fe00e10a978a6f8d701535a25e7a9e

Aryl hydrocarbon receptor (AHR) ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) cause altered cell proliferation in many tissues in vivo and cell types in vitro, and the AHR has been suggested to play a role in cell cycle regulation in mammalian systems. However, the mechanisms underlying these effects are poorly understood. The overall objective of the present work was to investigate possible interactions between cell proliferation, the cell cycle, and AHR signal transduction in a piscine system, the PLHC-1 cell line, which is being used increasingly in
aquatic toxicological research. The specific objectives were to characterize proliferation rates and the cell cycle in these cells, to measure effects of TCDD on cell proliferation, and to determine if expression of the AHR varies during the cell cycle. The doubling time of PLHC-1 cells was determined to be 22 h, and the durations of the G1, S and G2/M stages of the cell cycle were 13, 3, and 6 h, respectively. A minimum seeding density of 1.2 x 10^5 cells/cm^2 in medium with 10% calf serum and 0.3 x 10^5 cells/cm^2 in 10% fetal bovine serum was found to be required for subsequent proliferation. Of several cell cycle inhibitors tested, only aphidicolin and nocodazole were effective for obtaining synchronous cell populations. TCDD was found to inhibit PLHC-1 cell proliferation in a time- and dose-dependent manner in multiple passages of one sub-clone, but not in several other sub-clones. Neither AHR mRNA nor protein expression varied during the cell cycle, as measured by RT-PCR and specific binding of [3H]TCDD in synchronous PLHC-1 cells. This work establishes techniques for identifying and characterizing possible interactions between the cell cycle and AHR signal transduction in PLHC-1 cells. Taken together, the results indicate that PLHC-1 cells are amenable to analysis of AHR-cell cycle interactions, but that heterogeneity of sub-clones may complicate their use for investigating AHR-mediated changes in proliferation.


http://www.sciencedirect.com/science/article/B6T4G-45SH8MV-1/2/c75f29ce849159502176d569dd0867d0

In order to monitor the influence of estrogenic compounds on the reproductive physiology of fish, molecular markers for zebrafish vitellogenin, estrogen receptor and ZP2 were developed. For this purpose, sequence information about the zebrafish estrogen receptor and vitellogenin had to be obtained. By means of RT-PCR, a sequence fragment of the zebrafish estrogen receptor [alpha] was cloned and sequenced. Continuous cDNAs of two zebrafish vitellogenin-like gene products (zfvg1 and zfvg3) were constructed by the help of expressed sequence tags of zebrafish and completely sequenced. The sequences of the estrogen receptor and of the vitellogenins showed significant similarities to corresponding cDNAs of other fish species. Expression of these gene products was measured following exposure to 17[alpha]-ethinylestradiol and compared with histological endpoints. RT-PCR was used as a semiquantitative technique to record gene expression in adult male zebrafish, which were exposed to 17[alpha]-ethinylestradiol in time-and dose-response experiments. As for time-dependent expression, all hepatic genes investigated were expressed at considerable amounts from 24 h after onset of exposure to 50 ng/l 17[alpha]-ethinylestradiol to the end of experiment (17 days). In testes, expression of the estrogen receptor-as well as ZP2-mRNA remained unchanged for the entire experiment, except for the individuals exposed for 17 days, which displayed elevated expression levels of ZP2. In the dose-response experiment, male zebrafish were exposed to 17[alpha]-ethinylestradiol in concentrations from 0.25-85 ng/l for 4 and 21 days. LOECs for vitellogenin as well as estrogen receptor [alpha] expression were found to be 2.5 ng/l already after 4 d of exposure. Extension of the exposure time to 21 days resulted in enhanced transcription of vitellogenin-mRNAs at 2.5 ng/l 17[alpha]-ethinylestradiol, whereas the detection limit could not be lowered. In contrast, in testes no induction of both ZP2 as well as estrogen receptor expression was detected at any concentration tested. To examine estrogen-caused alterations at the ultrastructural level, liver and testes of males exposed to 25 ng/l 17[alpha]-ethinylestradiol were analysed. Male livers responded with a feminisation reflected by the proliferation of rough endoplasmatic reticulum and Golgi apparatus typical of female hepatocytes during vitellogenesis. However, in testes no signs of feminisation were detectable; rather, destructive phenomena like phagocytosis of sperm cells by Sertoli cells were observed. Thus, in sexually differentiated males no reorganisation of the gonadal tissue towards an ovary could be definitely detected at any level investigated.

http://www.sciencedirect.com/science/article/B6T4G-41306X9-3/2/786cf1ad36e62713f06046f743007f8a

The present study examined the interrelationship of GSH depletion, apoptosis, mutation, and cell proliferation following carcinogen exposure. Medaka (Oryzias latipes) were investigated following a 28 day, three times/week pulse exposure to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Fish (5 weeks old) were exposed to MNNG at concentrations of 0, 0.5, and 1 mg l-1 and reared for 3, 5 and 7 more months after the last day of exposure. GSH levels were decreased in the higher concentration groups and longer-reared groups. Flow cytometric analysis revealed that fish from the groups reared 3 and 5 months showed active apoptotic changes in the dose- and time-dependent manner, but the group reared 7 months had fewer apoptotic, rather showed more necrotic and carcinogenic alterations. Mutational responses were detected by an arbitrarily primed polymerase chain reaction (AP-PCR) fingerprinting method using whole body DNA samples as templates and pBR primer. A mutational change was expressed by a loss or gain of a band. There was a time-dependent mutational change, but no distinctive concentration-dependent one. A band from normal fish sample that disappeared after treatment of MNNG was excised and sequenced. The band had an 869 base pair-long sequence, however, there was no putative protein-coding region based on an analysis by DNAsis. Spindle cell sarcomas invading muscle were detected on the whole body sections from three of ten fish examined, and immunohistochemical analysis with PCNA showed that tumor cells were actively proliferating. However, terminal deoxynucleotidyl transferase (TdT) assay showed that tumored fish still had active apoptotic cell changes in the tissues without tumor. This study shows not only the interrelationship of GSH depletion, apoptosis, mutation and cell proliferation, but also indicates that medaka is appropriate as a fish model for research on the passage of carcinogenesis.


http://www.sciencedirect.com/science/article/B6T4G-4BRBFGM-2/2/5babab68165d69b7827042a1b9f8c3604

Metallothionein (MT) has been used widely as a potential molecular marker to detect the deleterious effects of heavy metals in aquatic ecosystem. Here we exposed ayu, Plecoglossus altivelis, to zinc (Zn) and tested the distribution as well as the induction of MT in various tissues such as liver, kidney, intestine and stomach. MT induction was significant in liver tissue, followed by kidney and intestine, whereas no induction was detected in stomach. The gene encoding ayu MT was successfully cloned and characterized. Complete nucleotide sequencing and analysis of the 4.5 kb DNA fragment containing the ayu MT gene revealed that the gene has three exons interrupted by two introns, a 5'-flanking region of about 2.5 kb and about 1.6 kb of 3'-flanking region. In grouper heart and kidney cells, the 2.5 kb promoter containing eight metal responsive elements (MREs), two hepatic nuclear factor 5 responsive elements (HNF5REs) and one cAMP responsive element (CRE) had the highest reporter activity.

Chemosynthetic mussels were collected in the vicinity of gas and petroleum seeps in the Gulf of Mexico. Aryl hydrocarbon hydroxylase (AHH) and glutathione S-transferase (GST) activities in the hepatopancreas and gill, respectively, were elevated in mussels collected at the site more highly contaminated with polynuclear aromatic hydrocarbons (PAHs). The aryl hydrocarbon receptor (AhR) and the 4S PAH binding protein (PBP) are ligand-activated transcription factors which regulate expression of various genes including cytochrome P450 1A. The presence of these proteins was investigated in PAH-exposed mussels. RT-PCR analysis revealed only a 45.2% nucleotide similarity between the AhR2 from Fundulus heteroclitus and from mussel mRNA transcripts containing a putative member of the PAS gene family. Furthermore, in gel electrophoretic mobility shift and protein cross-linking assays utilizing mussel cytosol which was incubated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), no specifically-bound retarded band with a [32P]dioxin responsive element was clearly indicated. Likewise, sucrose density gradient analysis of cytosol incubated with [3H]TCDD did not give a specifically-bound 8-10S peak associated with the AhR complex. In contrast, incubation of mussel cytosol with [3H]benzo(a)pyrene (BaP) gave a 4S peak which was not displaced by TCDD but was nonspecifically decreased by excess BaP or benzo(ghi)perylene. The potential role of the 4S PAH binding protein in the induction of CYP1A-dependent activity in these species is currently unclear. Mussels collected from the North Sea were treated with BaP (5 mg/kg) or TCDD (20 μg/kg) for 48 h to investigate induction of enzyme activities in mussels from a pristine location. Western blot analysis indicated the presence of a 33 kDa protein when a PAH binding protein antibody was used, but no detectable induction of AHH or GST activity was observed in the treated mussels.

Arch Dermatol (1)


Context Beginning in 1957, patients have been described with localized alopecia characterized histopathologically by mucin deposition within hair follicles (follicular mucinosis [FM]). At least 2 distinct diagnostic entities have been proposed: one occurring in children and young adults without association with other diseases ("idiopathic" FM), the other occurring in elderly patients and associated with mycosis fungoides or Sezary syndrome ("lymphoma-associated" FM). Objective To determine whether idiopathic and lymphoma-associated FM are distinct or related entities. Design Case series. Setting Department of Dermatology, University of Graz, Graz, Austria. Patients Forty-four patients with FM were divided into 2 groups. Group 1 comprised 16 patients (mean age, 37.5 years) with no associated mycosis fungoides or Sezary syndrome; group 2 was made up of the other 28 (mean age, 52.2 years), who had clinicopathologic evidence of cutaneous T-cell lymphoma. Results Mean age was lower in patients with idiopathic FM, but a considerable overlapping among the 2 groups was present. Location on the head and neck region was common in both groups, but most patients with lymphoma-associated FM had lesions also on other body sites. In fact, solitary lesions at presentation were common in patients with idiopathic FM (11 [68.8%] of 16 patients), but uncommon in those with lymphoma-associated FM (2 [7.1%] of 28 patients). Histopathologic findings did not allow clear-cut differentiation of the 2
Finally, a monoclonal rearrangement of the T-cell receptor \((\text{gamma})\) gene was demonstrated by polymerase chain reaction analysis in about 50% of tested cases from each group. Conclusions Criteria previously reported to differentiate idiopathic from lymphoma-associated FM proved ineffective. In analogy to localized pagetoid reticulosis (Woringer-Kolopp disease), small-plaque parapsoriasis, and so-called solitary mycosis fungoides, idiopathic FM may represent a form of localized cutaneous T-cell lymphoma.

**Arch Gen Psychiatry**  (2)


http://archpsyc.ama-assn.org/cgi/content/abstract/61/2/177

Background Substantial evidence supports a role for dysfunction of the serotonin transporter in the pathogenesis of major depression. Several studies have found reciprocal interactions between the serotonergic system and both brain-derived neurotrophic factor and glutamate, which are known to modulate or affect hippocampal morphologic characteristics. Objective To examine the influence of a polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene on hippocampal volumes in patients with major depression and healthy controls. Design Baseline investigation of a prospective magnetic resonance imaging study with a 4-year follow-up period. Patients We examined 40 inpatients with major depression as well as 40 healthy controls matched for age, sex, and handedness. Main Outcome Measures Subjects underwent high-resolution magnetic resonance imaging. Furthermore, genotyping for the 5-HTTLPR biallelic polymorphism was performed, which consists of a 44-base pair insertion (L allele) or deletion (S allele). Results Patients with the L/L homozygous genotype had significantly smaller hippocampal gray matter (left hemisphere: \(P = .003\); right hemisphere: \(P = .01\)) and white matter volumes (left hemisphere: \(P = .001\); right hemisphere: \(P = .002\)) than controls with this genotype. No significant differences were found between patients and controls with the L/S or S/S genotype. Moreover, patients with the L/L genotype had significantly smaller hippocampal white matter volumes than those with the L/S or S/S genotype (\(P = .03\)). Conclusions These findings suggest that homozygosity for the L allele is associated with decreased hippocampal volumes in patients with major depression but not in healthy controls. A possible explanation is that the interaction between the serotonergic system and neurotrophic factors as well as excitatory amino acid neurotransmission may affect hippocampal morphologic characteristics.


http://archpsyc.ama-assn.org/cgi/content/abstract/59/7/613

Background Evidence suggests that serotonin transporter gene promoter polymorphism (5HTTLPR)-dependent low transcriptional activity of the human serotonin transporter gene may be a genetic susceptibility factor for depression. We studied the behavioral responses to
tryptophan depletion (TD) in healthy women with and without a first-degree family history of depression and examined the relationship to 5HTTLPR alleles. Methods Twenty-four healthy women with a negative family history of depression and 21 women with a positive family history of depression were genotyped for the polymorphism of the 5HTTLPR and then entered a double-blind, placebo-controlled, randomized crossover TD study. The effects of these interventions were assessed with measures of depression and plasma tryptophan levels. Results The TD induced a robust decrease of plasma tryptophan levels in all women irrespective of family history of depression or 5HTTLPR genotypes. The s/s genotype of the 5HTTLPR was associated with an increased risk of developing depressive symptoms during TD irrespective of family history. In contrast, individuals with the l/l genotype did not develop depressive symptoms, irrespective of family history. Finally, s/l subjects without family history showed a mood response that was intermediate between the s/s and l/l subjects, while s/l subjects with a family history of depression showed the same depressiogenic effect of TD as seen in the s/s subjects. Conclusions The results of the present study suggest that the s-allele of the 5HTTLPR and a positive family history of depression are additive risk factors for the development of depression during TD.

Arch Ophthalmol (4)


http://archopht.ama-assn.org/cgi/content/abstract/120/11/1534

Objective To validate a real-time polymerase chain reaction (PCR) assay allowing rapid and sensitive detection and quantitation of 4 common infectious posterior uveitis pathogens. Methods A real-time PCR assay using previously validated primer sets for cytomegalovirus, herpes simplex virus, varicella-zoster virus, and Toxoplasma gondii was developed. A standard curve for quantitation of pathogen load was generated for each pathogen using SYBR Green I fluorescence detection. Ocular samples from patients with posterior uveitis and from negative control samples were assayed and compared with standards to identify pathogens and quantify infectious load. Results Sensitivity for detection of purified pathogen DNA by PCR was not reduced by application of the real-time method. Standard curves for the quantitation of pathogen loads showed sensitivity to fewer than 10 organisms for all pathogens. The technique was applied to 2 clinical problems. First, sensitivities of existing monoplex and multiplex PCR were compared by real-time PCR. No significant difference in sensitivity was observed between multiplex and monoplex techniques. Second, pathogen loads of vitreous specimens from patients previously diagnosed as having infectious posterior uveitis were calculated. Pathogen loads were found to be generally higher for patients with disease caused by varicella-zoster virus than those caused by cytomegalovirus or herpes simplex virus. Conclusions Real-time PCR may be applied to infectious agents responsible for posterior uveitis. This technique will likely prove useful for the diagnosis of posterior uveitis as well as the linkage of pathogen to disease. Clinical Relevance Real-time PCR provides a rapid technique for quantitatively evaluating ocular samples for the presence of infectious pathogens.

Objective To describe retinal and optic disc atrophy and a progressive decrease of visual function in 2 Japanese brothers. Both had a mutation in the CACNA1F gene, the causative gene of incomplete congenital stationary night blindness (CSNB). Methods We studied observational case reports and performed comprehensive ophthalmologic examinations including best-corrected visual acuity, biomicroscopy, ophthalmoscopy, fundus photography, and electroretinography. Genomic DNA was extracted from the peripheral blood, and all 48 exons of the CACNA1F gene were directly sequenced. Results The 2 brothers had retinal and optic disc atrophy and a progressive reduction of visual acuity with increasing age. Although these clinical features are not typical of previous patients with incomplete CSNB, both patients had an in-frame mutation with deletion and insertion in exon 4 of the CACNA1F gene. In both patients, the bright-flash, mixed rod-cone electroretinogram had a negative configuration, a characteristic of incomplete CSNB. However, the full-field scotopic and photopic electroretinograms were nonrecordable, indicating severe, diffuse retinal malfunction, which is not typical in incomplete CSNB. Conclusion These findings indicate that a mutation of the CACNA1F gene may be associated with retinal and optic disc atrophy with a progressive decline of visual function. Clinical Relevance In patients with retinal and optic disc atrophy associated with negative-type electroretinograms, a CACNA1F gene mutation should be considered.


Objectives To establish that the protozoan Acanthamoeba is one of the causative organisms associated with non-contact lens-related keratitis in the Indian population and to develop a simple and sensitive diagnostic assay for clinical testing. Design DNA sequencing of nuclear 18S and 26S ribosomal DNA motifs was performed and compared with the reference Acanthamoeba strains, to establish the genetic identity of the putative amoeba isolates obtained from the corneal scrapings of non-contact lens-wearing patients with keratitis. Ribosomal DNA typing of clinical corneal scrapings from the patients with keratitis was performed by means of a simple agarose gel-based multiplex polymerase chain reaction assay, to detect the cases of Acanthamoeba keratitis. Results The ribosomal DNA analysis of 15 putative amoeba isolates obtained from the corneal scrapings of 14 patients with keratitis and 1 from the patients' environment established the isolates to be pathogenic forms of Acanthamoeba belonging to type T4 ribosomal DNA genotype. Multiplex polymerase chain reaction assay was specific and sensitive enough to detect as low as 5 pg of Acanthamoeba DNA. Its utility as a reliable diagnostic assay was demonstrated directly with the use of 34 additional corneal scrapings. Conclusions Acanthamoeba is one of the causative organisms of keratitis in Indian patients with no history of contact lens usage. Moreover, the Acanthamoeba infection can be easily detected in the clinical samples by means of the simple multiplex polymerase chain reaction assay based on ribosomal DNA typing. Clinical Relevance This study suggests the need and means to determine the incidence and prevalence of Acanthamoeba keratitis in India and elsewhere. Moreover, the polymerase chain reaction assay would help in early and definitive diagnosis, leading to better prognosis of Acanthamoeba keratitis condition.

Objective Bornholm eye disease (BED) consists of X-linked high myopia, high cylinder, optic nerve hypoplasia, reduced electroretinographic flicker with abnormal photopic responses, and deuteranopia. The disease maps to chromosome Xq28 and is the first designated high-grade myopia locus (MYP1). We studied a second family from Minnesota with a similar X-linked phenotype, also of Danish descent. All affected males had protanopia instead of deuteranopia. Methods X chromosome genotyping, fine-point mapping, and haplotype analysis of the DNA from 22 Minnesota family individuals (8 affected males and 5 carrier females) and 6 members of the original family with BED were performed. Haplotype comparisons and mutation screening of the red-green cone pigment gene array were performed on DNA from both kindreds. Results Significant maximum logarithm of odds scores of 3.38 and 3.11 at {theta} = 0.0 were obtained with polymorphic microsatellite markers DXS8106 and DXYS154, respectively, in the Minnesota family. Haplotype analysis defined an interval of 34.4 cM at chromosome Xq27.3-Xq28. Affected males had a red-green pigment hybrid gene consistent with protanopia. We genotyped Xq27-28 polymorphic markers of the family with BED, and narrowed the critical interval to 6.8 cM. The haplotypes of the affected individuals were different from those of the Minnesota pedigree. Bornholm eye disease-affected individuals showed the presence of a green-red hybrid gene consistent with deuteranopia. Conclusions Because of the close geographic origin of the 2 families, we expected affected individuals to have the same haplotype in the vicinity of the same mutation. Mapping studies, however, suggested independent mutations of the same gene. The red-green and green-red hybrid genes are common X-linked color vision defects, and thus are unrelated to the high myopia and other eye abnormalities in these 2 families. Clinical Relevance X-linked high myopia with possible cone dysfunction has been mapped to chromosome Xq28 with intervals of 34.4 and 6.8 centimorgan for 2 families of Danish origin.

Arch Otolaryngol Head Neck Surg (2)


http://archotol.ama-assn.org/cgi/content/abstract/130/1/78

Objective To assess alcohol dehydrogenase 3 (ADH3) polymorphism at position Ile349Val as indicator of risk factor for upper aerodigestive tract (UADT) cancer to verify its association with UADT cancer in nonalcoholic or nonsmoking individuals. Design Cross-sectional study. Setting Primary care or referral center. Patients The study group consisted of 141 consecutive patients with newly diagnosed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx admitted for surgical treatment. The comparison group consisted of 94 inpatients without cancer from the A. C. Camargo or other Sao Paulo (Brazil) hospital and 40 healthy individuals. Intervention All participants were interviewed and data were collected using a structured questionnaire. After written informed consent was obtained, 20 mL of blood was collected in heparinized tubes. Main Outcome Measures Odds ratio for ADH3 genotypes using logistic regression models. Results After adjustment for sex, age, tobacco use, and history of cancer in first-degree family relatives, a significantly higher odds ratio for UADT cancer was observed among individuals with AA genotype and low cumulative alcohol consumption ([<=]100 kg of ethanol) (odds ratio = 3.8 [95% confidence interval, 1.5-9.7]). A 4-fold increase in odds ratio for UADT cancer among individuals with AA genotype and low tobacco consumption ([<=]25 pack-years) was also found in the adjusted model. Conclusions These results suggest that genotype AA may be a risk factor for UADT cancer, especially in individuals with low alcohol or tobacco consumption. However, further epidemiological case-control or cohort studies, preferably prospective, are needed to establish the exact role of ADH3 polymorphism and its association
with the development of UADT cancers.


Objectives To examine the association between cyclooxygenase-2 (COX-2) expression with epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), and latent membrane protein 1 (LMP-1) expression and with COX-2 promoter methylation status in primary nasopharyngeal cancer (NPC) tumors and to determine COX-2 promoter methylation status in NPC cell lines. Design Retrospective study. Setting Patients with NPC were referred to the Department of Otolaryngology-Head and Neck Surgery for treatment. Patients Formalin-fixed, paraffin-embedded NPC specimens from 42 patients were obtained. Interventions Immunohistochemical expression of COX-2, EGFR, VEGF, iNOS, and LMP-1 was performed in 42 NPC samples. COX-2 promoter methylation status was studied in 20 separate specimens and in 4 NPC cell lines. Main Outcome Measures (1) COX-2, EGFR, VEGF, iNOS, and LMP-1 expression; and (2) COX-2 promotor methylation status. Results COX-2 was overexpressed in 79% of NPC specimens and was associated with EGFR status (P = .03) but not with LMP-1 or iNOS. In primary NPC tissue, methylation of the COX-2 promoter was seen in 4 of 7 COX-2-negative and 1 of 13 COX-2-positive immunohistochemical cases. COX-2 promoter methylation was found in the CNE-1 cell line. Conclusions Nasopharyngeal cancer may be a useful target for selective COX-2 inhibition. The absence of promoter methylation may be a necessary component of COX-2 overexpression, and promoter methylation may be one of the mechanisms that regulate COX-2 expression.


Objective To investigate whether interleukin 1{beta} (IL-1{beta}) exon 5 and IL-1 receptor antagonist (IL-1Ra) gene polymorphisms can be used as markers of susceptibility to febrile convulsions in children. Methods Children were divided into 2 groups: those with febrile convulsions (group 1; n = 51) and normal control subjects (group 2; n = 83). Polymorphisms for IL-1{beta} exon 5 and IL-1Ra gene polymorphisms were detected by polymerase chain reaction. Genotypes and allelic frequencies for IL-1{beta} exon 5 and IL-1Ra gene polymorphisms in both groups were compared. Results Genotype and allele frequencies for IL-1{beta} exon 5 in both groups were not significantly different. Proportions of E1 homozygotes and E1/E2 heterozygotes for IL-1{beta} exon 5 were 50 (98.1%) and 1 (1.9%), respectively, in group 1 and 82 (98.8%) and 1 (1.2%), respectively, in group 2. Frequencies of alleles E1 and E2 for IL-1{beta} exon 5 were 101 (99.0%) and 1 (1.0%), respectively, in group 1 and 165 (99.4%) and 1 (0.6%), respectively, in group 2. Genotype proportions and allele frequencies for IL-1Ra between groups were
significantly different. Proportions of genotypes I/I and I/II for IL-1Ra were 49 (96.1%) and 2
(3.9%) in group 1 and 69 (83.1%) and 14 (16.9%) in group 2. Frequencies of alleles I and II for IL-
1Ra were 100 (98.0%) and 2 (2.0%) in group 1 and 152 (91.6%) and 14 (8.4%) in group 2.
Conclusions The IL-1Ra allele I is associated with a higher susceptibility to febrile convulsion,
which may become a useful marker for predicting the development of febrile convulsions. The IL-
1{beta} exon 5 gene polymorphisms are not a useful marker for predicting the susceptibility to
febrile convulsions.

Arch Surg (2)


http://archsurg.ama-assn.org/cgi/content/abstract/138/3/291

Hypothesis The cause of breast cancer is linked to many macroscopic events, including benign
breast disease. In this study we asked whether molecular changes could discriminate
fibroadenoma, which is one of the most common benign breast disease lesions associated or not
Subjects Archival tissues in 32 cases of fibroadenoma, diagnosed in the same breast as a breast
carcinoma, are compared with a control group of 26 cases of fibroadenomas unaffected by breast
cancer. Main Outcome Measures Histological features are characterized in all samples. The
epithelial and stromal components are analyzed for a loss of heterozygosity and a microsatellite
instability using a polymerase chain reaction-based method with 11 polymorphic microsatellite
markers at 7 chromosomal regions frequently altered in breast cancer. The p53 gene mutations
were also determined at exons 5 to 9. Results The frequency of complex fibroadenomas was
similar in both groups (P =.42). Only in the case group did we observe proliferative lesions
confined in fibroadenomas, including atypical ductal hyperplasia (2 cases), lobular neoplasia (3
cases), or low-grade ductal carcinoma in situ (2 cases). There is no significant morphological
difference between the 2 groups. Neither microsatellite alterations nor p53 gene mutations are
present in the fibroadenoma components. Loss of heterozygosity is found only in the epithelial
component of the 2 ductal carcinomas in situ confined in fibroadenomas. Conclusions Genetic
alterations, which are most frequently involved in malignant breast carcinomas, are not present in
fibroadenomas, regardless of their association with breast cancer or their histological complexity.
These findings suggest that fibroadenomas are not associated with breast carcinogenesis.

Reflux Disease Influences Cyclooxygenase-2 Gene Expression in the Squamous Epithelium of

http://archsurg.ama-assn.org/cgi/content/abstract/139/7/712

Hypothesis Although genetic changes associated with the progression to Barrett esophagus and
adenocarcinoma have been identified, changes in gene expression associated with
gastroesophageal reflux disease have not been reported. We examined expression levels of
several genes important in carcinogenesis and compared expression levels with alterations in
esophageal acid exposure. Patients, Design, and Setting Prospective analysis of 61 patients
initially seen with reflux symptoms at a private academic hospital. Interventions Paired esophageal biopsy specimens of squamous epithelium 3 cm above the squamocolumnar junction. All patients had 24-hour pH monitoring performed. Main Outcome Measures Cyclooxygenase (COX) 1, COX-2, thymidylate synthase, human telomerase reverse transcriptase (hTERT), Bcl-2 protein, survivin protein, secreted protein acidic and rich in cysteine (SPARC), tetraspan (TSPAN), and caudal-type homeobox transcription factor 2 (CDX2) messenger RNA expression analysis was performed on snap-frozen, microdissected tissue using a quantitative reverse transcriptase-polymerase chain reaction method. Linear regression and the Pearson product moment correlation were used to relate gene expression to parameters of the 24-hour pH record. Results Expression levels of COX-2 correlated positively with the 24-hour pH score (r = 0.25, P =.05). There was no correlation between the expression of other tested genes and esophageal acid exposure. There was also no significant increase in COX-2 expression in patients with esophagitis or in those who used nonsteroidal anti-inflammatory drugs. Conclusions To our knowledge, these data provide among the first reported correlation of genetic changes and increased esophageal acid exposure in patients with gastroesophageal reflux symptoms. The changes in gene expression occur before any metaplastic changes in the tissue are apparent, and may in the future be useful in predicting which patients will progress through a metaplasia-dysplasia carcinoma sequence.

Archives of Biochemistry and Biophysics (14)


Comparison between the cDNA sequence of CYP4A11 and that deduced from a published genomic clone suggested the presence of an additional CYP4A gene in humans, CYP4A22. PCR amplification of genomic DNA yielded overlapping clones covering 13 kb of genomic DNA and extending from 1003 bp upstream from CYP4A11 translation initiation to 135 bp upstream of the mRNA polyadenylation signal. Sequence and Southern blot analysis showed the presence in humans of two highly homologous CYP4A genes, CYP4A11 and CYP4A22. These two genes share 96% sequence identity and have similar intron/exon sizes and distribution. Short nucleotide insertions ([les]10 bp) in introns 1, 3, 9, and 11, and deletions ([les]18 bp) in introns 4, 6, and 11 differentiate the two genes. RT-PCR amplification of human kidney RNA followed by restriction fragment analysis showed that CYP4A11 is the predominant isoform expressed in kidney.


We have generated site-specific mutants of the kringle 2 domain of tissue-type plasminogen activator ([K2tPA]) in order to identify directly the cationic center of the protein that is responsible
for its interaction with the carboxyl group of important [omega]-amino acid effector molecules, such as [epsi]-amino caproic acid (EACA). Molecular modeling of [K2tPA], docked with EACA, based on crystal structures of the kringle 2 region of prothrombin and the kringle 4 domain of human plasminogen, clearly shows that Lys33 is the only positively charged amino acid in [K2tPA] that is sufficiently proximal to the carboxyl group of the ligand to stabilize this interaction. In order to examine directly the importance of this particular amino acid residue in this interaction, we have constructed, expressed, and purified three recombinant (r) mutants of [K2tPA], viz., Lys33Thr, Lys33Leu, and Lys33Arg, and found that only the last variant retained significant ability to interact with EACA and several of its structural analogues at neutral pH. In addition, another mutated r-[K2tPA], i.e., Lys33His, interacts very weakly with [omega]-amino acids at neutral pH and much more strongly at lower pH values where His33 would be expected to undergo protonation. This demonstrates that any positively charged amino acid at position 33 satisfies the requirement for mediation of significant bindings to this class of molecules. Since, in other kringles, positively charged residues at amino acid sequence positions homologous to Lys68, Arg70, and Arg71 of [K2tPA] have been found to participate in kringle interactions with EACA-like compounds, we have also examined the binding of EACA, and some of its analogues, to three additional r-[K2tPA] variants, i.e., Lys68Ala, Arg70Ala, and Arg71Ala. In each case, binding of these [omega]-amino acids to the variant kringles was observed, with only the Lys68Ala variant showing a slightly diminished capacity for this interaction. These investigations provide clear and direct evidence that Lys33 is the principal cationic site in wildtype r-[K2tPA] that directly interacts with the carboxyl group of [omega]-amino acid effector molecules.


http://www.sciencedirect.com/science/article/B6WB5-4C7VYWS-6/2/ec69c0f7bb9127223e32380e87e04608

Two UDP-glucuronosyltransferases (UGT2B9*2 and UGT2B33) have been isolated from female rhesus monkey liver. Microsomal preparations of the cell lines expressing the UGTs catalyzed the glucuronidation of the general substrate 7-hydroxy-4-(trifluoromethyl)coumarin in addition to selected estrogens ([beta]-estradiol and estriol) and opioids (morphine, naloxone, and naltrexone). UGT2B9*2 displayed highest efficiency for [beta]-estradiol-17-glucuronide production and did not catalyze the glucuronidation of naltrexone. UGT2B33 displayed highest efficiency for estriol and did not catalyze the glucuronidation of [beta]-estradiol. UGT2B9*2 was found also to catalyze the glucuronidation of 4-hydroxyestrone, 16-epiestriol, and hyodeoxycholic acid, while UGT2B33 was capable of conjugating 4-hydroxyestrone, androsterone, diclofenac, and hyodeoxycholic acid. Three glucocorticoids (cortisone, cortisol, and corticosterone) were not substrates for glucuronidation by liver or kidney microsomes or any expressed UGTs. Our current data suggest the use of [beta]-estradiol-3-glucuronidation, [beta]-estradiol-17-glucuronidation, and estriol-17-glucuronidation to assay UGT1A01, UGT2B9*2, and UGT2B33 activity in rhesus liver microsomes, respectively.


http://www.sciencedirect.com/science/article/B6WB5-4DPBYHV-KG/2/97c9162916a35bfd49ea186d26556ee3

A novel fusion protein expression plasmid that allows ready purification and subsequent facile release of the target molecule has been constructed and employed to express in Escherichia coli
and purify the tissue plasminogen activator kringle 1 domain ([K1tPA] residues C92---C173). The resulting plasmid encodes the tight lysinebinding kringle (K)1 domain of human plasminogen ([K1HPg]) followed by a peptide (PfXa) containing a factor Xa-sensitive bond, downstream of which [K1tPA] was inserted. The recombinant (r) [K1HPg]PfXa[K1tPA] fusion polypeptide was purified from various cell fractions in one step by Sepharose-lysine affinity chromatography. After cleavage with fXa, the mixture was repassaged over Sepharose-lysine, whereupon the r-[K1tPA]-containing polypeptide passed unretarded through the column. A homogeneous preparation of this material was then obtained after a simple step employing fast protein liquid chromatography. The purified r-[K1tPA], which contained the amino acid sequence SNAS[K1tPA]S, provided an amino-terminal amino acid sequence, through at least 20 amino acid residues, that was identical to that predicted from the cDNA sequence. The molecular mass of r-SNAS[K1tPA]S, determined by electrospray mass spectrometry, was 921.9 +/- 4.0 (expected molecular mass, 9623.65). 1H-NMR spectroscopy and thermal stability studies of r-SNAS[K1tPA]S revealed that the purified material was properly folded and similar to other isolated kringle domains. Additionally, employment of this methodology revealed that only a very weak interaction between [epsilon]-aminocaproic acid and the isolated r-[K1tPA] domain occurred.


http://www.sciencedirect.com/science/article/B6WB5-4DN9YRM-14K/2/96fa446a666d5d47471e3ba8f10c15e

A mammalian cell expression plasmid containing cytochrome P450IIIA7 complementary DNA was constructed. Breast cancer cells (MCF-7) were transfected with the plasmid and neomycin-resistant selection marker plasmid. We established three cell lines, termed M13, M21, and M27, which expressed the cytochrome P450IIIA7 as examined by RNA blot and immunoblot analyses. These cell lines showed 8- to 10-fold higher sensitivity against aflatoxin B1 compared to parental MCF-7 cells, suggesting that cytochromes P450IIIA7 expressed in the cells were responsible for the production of the cytotoxic metabolite of aflatoxin B1.


http://www.sciencedirect.com/science/article/B6WB5-47HS441-D/2/1d1e70368b93501e212acce0bee71198

Amyloid precursor protein (APP) cleaving enzyme (BACE) is the enzyme responsible for [beta]-site cleavage of APP, leading to the formation of the amyloid-[beta] peptide that is thought to be pathogenic in Alzheimer's disease (AD). Hence, BACE is an attractive pharmacological target, and numerous research groups have begun searching for potent and selective inhibitors of this enzyme as a potential mechanism for therapeutic intervention in AD. The mature enzyme is composed of a globular catalytic domain that is N-linked glycosylated in mammalian cells, a single transmembrane helix that anchors the enzyme to an intracellular membrane, and a short C-terminal domain that extends outside the phospholipid bilayer of the membrane. Here we have compared the substrate and active site-directed inhibitor binding properties of several recombinant constructs of human BACE. The constructs studied here address the importance of catalytic domain glycosylation state, inclusion of domains other than the catalytic domain, and incorporation into a membrane bilayer on the interactions of the enzyme active site with peptidic ligands. We find no significant differences in ligand binding properties among these various
constructs. These data demonstrate that the nonglycosylated, soluble catalytic domain of BACE faithfully reflects the ligand binding properties of the full-length mature enzyme in its natural membrane environment. Thus, the use of the nonglycosylated, soluble catalytic domain of BACE is appropriate for studies aimed at understanding the determinants of ligand recognition by the enzyme active site.


http://www.sciencedirect.com/science/article/B6WB5-4DM296T-1/2/c183bfec6ed10071ffdd420532fb0bea

Microarray technology has been used to discover 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) induced gene expression changes in rat small intestine in vivo. Here, we report gene expression changes related to intestinal absorption or transport, the immune system and angiogenesis in response to 1,25-(OH)2D3. Vitamin D deficient rats were intrajugularly given vehicle or vehicle containing 730 ng of 1,25-(OH)2D3/kg of body weight. Intestinal mRNA was harvested from duodenal mucosa at 15 min, 1, 3, and 6 h post-injection and studied by Affymetrix microarrays. Genes significantly affected by 1,25-(OH)2D3 were confirmed by quantitative RT-PCR with remarkable agreement. The most strongly affected gene in intestine was CYP24 with 97-fold increase at 6 h post-1,25-(OH)2D3 treatment. Intestinal calcium absorption genes: TRPV5, TRPV6, calbindin D9k, and Ca2+ dependent ATPase all were up-regulated in response to 1,25-(OH)2D3, supporting the currently accepted mechanism of 1,25-(OH)2D3 induced transcellular calcium transport. However, a 1,25-(OH)2D3 suppression of several intra-/intercellular matrix modeling proteins such as sodium/potassium ATPase, claudin 3, aquaporin 8, cadherin 17, and RhoA suggests a vitamin D regulation of tight junction permeability and paracellular calcium transport. Several other genes related to the immune system and angiogenesis whose expression was changed in response to 1,25-(OH)2D3 provided evidence for an immunomodulatory and anti-angiogenic role of 1,25-(OH)2D3.


http://www.sciencedirect.com/science/article/B6WB5-4C47K8J-1/2/02e3b43971f5975a27f9563b183350bea

We previously reported that galactosylceramide expression factor-1 (GEF-1), a rat homolog of hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs/Hgs), induces galactosylceramide and/or sulfatide expression and morphological changes in epithelial cells. Here, we show that GEF-1 induces myogenesis in MDCK and C3H10T1/2 cells. GEF-1 overexpression in MDCK cells (MDCK/GEF-1) appeared to promote trans-differentiation to myoblasts that expressed MyoD and myosin heavy chain (MHC). MDCK/GEF-1 cells also expressed several DNA-binding proteins (MyoD and MEF-2) that are essential for myogenesis. These results suggest that GEF-1 induces MDCK cells to enter an early stage of myogenesis. Subsequently, we tested whether GEF-1 could induce myogenesis in C3H10T1/2 mouse fibroblasts, which have the potential to differentiate into myoblast-like cells. Indeed, GEF-1 induced morphological changes that were consistent with myoblast-like cells, and both MyoD and MHC were expressed. Our results suggest that GEF-1 may induce MDCK and C3H10T1/2 cells to trans-differentiate into myoblast-like cells.

http://www.sciencedirect.com/science/article/B6WB5-46271BS-F/2/2851991d283833a1be504437a237f34c


http://www.sciencedirect.com/science/article/B6WB5-4DPC4KM-1PY/2/d43ba3aef6d608fc934185fb054efc095

The gene for rat cystathionine [beta]-synthase consists of 17 exons. Its transcripts are alternatively spliced, forming four distinct mRNA species. Type III consists of exons 1 through 12, 14, 15, and 17; type I also contains exon 16. The open reading frame of type IV spans exons 1 -> 13; type II, 3 -> 13. We cloned the corresponding cDNAs into appropriate expression vectors and inserted the constructs into Escherichia coli (I, III, and IV) and Chinese hamster (CH) cells (I through IV); all sequences were transcribed and translated. Catalytic activity was observed only for types I and III in lysates of transfected CH cells and transformed E. coli. The catalytic and kinetic properties of I and III were identical despite their structural difference (exon 16). Both isoforms exhibited 6 m Km constants for homocysteine which were reduced approximately eightfold by AdoMet; this elucidates the mechanism by which AdoMet regulates synthase activity. The four isoforms were differentially degraded by transfected cultured cells. Type III (t1/2 = 18 h) was degraded at the rate of type I (t1/2 = 6 h); thus the 14 amino acid residues encoded by exon 16 appear to enhance degradation of CBS. The half-lives of both types II and IV were markedly shorter (ca. 1 h). Western blots comparing rat liver to lysates from transfected CH cells revealed that hepatocytes express both isoforms. Type III was predominant, as predicted by its longer half-life and more abundant mRNA. PCR analysis of cDNA from various tissues revealed that type III mRNA was preferred in liver, kidney, and heart; equal amounts of I and III were found in brain.


http://www.sciencedirect.com/science/article/B6WB5-4DPC46C-1KP/2/db95e3f331a12cfff15a84c6cb6c26a64

A 1.3-kb rat testis cDNA clone for heme oxygenase-2 (HO-2) was used as a Northern blot hybridization probe, and a single homologous mRNA species, of approximately 1.3 kb in rabbit brain and testis was detected. This contrasted with the observation made with rat brain in which two HO-2 transcripts of approximately 1.3 and 1.9 kb were detected. Use of the same rat HO-2 probe to screen a rabbit brain cDNA library in [lambda]gt11 resulted in the recovery of a single 1.2-kb cDNA clone. This cDNA exhibits 84% overall nucleotide sequence homology with rat HO-2 and encodes a protein of 35,352 Da, displaying 88% amino acid sequence homology with rat testis HO-2. Furthermore, when expressed in Escherichia coli, the rabbit cDNA-encoded protein displays heme oxygenase activity and cross-reactivity with antibody to rat HO-2. Based on findings obtained through Western immunoblot analysis of partially purified HO-2 protein
prepared from rabbit testis and brain, the 35- to 36-kDa molecular form appears to be the major HO-2 form detected in the brain, whereas a 42-kDa species is the predominant form observed in rabbit testis. Having deduced the amino acid sequence of rabbit brain HO-2, we provide a comparison of this sequence with those of rat, mouse, and human HO-1 and rat HO-2, and thereby identify a 24-amino-acid-long peptide region which, except for one residue, is identical in all five species of HO-1 and HO-2 compared (96% similarity), and exhibits 100% similarity in predicted secondary structure (for this region) in all five proteins. We propose that this peptide may be important to the heme binding and isomer-specific tetrapyrrole cleavage activities of the heme oxygenase isozymes.


http://www.sciencedirect.com/science/article/B6WB5-46FVRR6-4/2/35f015c50ccd44a430c35b5a30bc7f32


http://www.sciencedirect.com/science/article/B6WB5-4700KV0-1/2/bbf4ff183e92158946c07d348996ca59

HepG2 cells that stably overexpress PPAR[alpha] were used to examine the regulation of the two known human CYP4A genes by Wy14643. Specific PCR amplification across intron 5 and restriction endonuclease analysis indicated that HepG2 cells possess genes corresponding to both the CYP4A11 cDNA and a more recently characterized gene, CYP4A22, that exhibits 95% identity to CYP4A11 in the coding region. These are unlikely to represent alleles because both genes were present in DNA samples from 100 of 100 individuals. Quantitative real-time PCR determined that CYP4A22 mRNA is expressed at significantly lower levels than CYP4A11 mRNA in human liver samples. The PPAR[alpha] agonist Wy14643 induced CYP4A11 mRNA in confluent cultures of HepG2 cells stably expressing the murine PPAR[alpha]-E282G mutant. This mutant exhibits a significantly decreased ligand-independent trans-activation and can be activated by Wy14643 to a level similar to that of wild-type PPAR[alpha]. Dexamethasone induced CYP4A11 mRNA in both control and PPAR[alpha]-E282G-expressing HepG2 cells, indicating that the induction of CYP4A11 by dexamethasone is independent of elevated PPAR[alpha] expression. Wy14643 or dexamethasone induction of CYP4A22 mRNA was not evident in either control or PPAR[alpha]-E282G-expressing HepG2 cells. The results indicate that CYP4A11 expression can be induced by glucocorticoids and peroxisome proliferators.


http://www.sciencedirect.com/science/article/B6WB5-46YBN84-4/2/dc6c61d40635a315381b64e2c803852d

Our aim was to determine the tissue distribution of CYP4F8, which occurs in human seminal vesicles and catalyzes 19-hydroxylation of prostaglandin H1 and H2 in vitro (J. Bylund, M.

Polyclonal antibodies were raised in rabbits against RVEPLG, the C-terminal end of CYP4F8, and purified by affinity chromatography. Screening of 50 human tissues for CYP4F8 immunoreactivity revealed protein expression, inter alia, in seminal vesicles, epidermis, hair follicles, sweat glands, corneal epithelium, proximal renal tubules, and epithelial linings of the gut and urinary tract. The CYP4F8 transcripts were detected by reverse transcription polymerase chain reaction and by Northern blot analysis. There was a prominent induction of CYP4F8 immunoreactivity and mRNA in psoriasis in comparison with unaffected epidermis of the same patients. The cDNA of CYP4F8 from plucked scalp hair roots was identical with the genital cDNA sequence. We conclude that CYP4F8 is present in epithelial linings and up regulated in epidermis of psoriatic lesions.

Archives of Internal Medicine (1)


http://archinte.ama-assn.org/cgi/content/abstract/165/4/409

Background Whether risk of invasive amebiasis due to Entamoeba histolytica is higher among human immunodeficiency virus (HIV)-infected persons than uninfected persons remains unclear, although intestinal colonization by Entamoeba dispar is common among men who have sex with men. Our objective was to determine the prevalence of invasive amebiasis and intestinal colonization by E histolytica and E dispar in HIV-infected persons and uninfected controls.

Methods We assessed the prevalence of invasive amebiasis by case review of 951 HIV-infected persons and by serologic studies of 634 of the 951 HIV-infected persons, 429 uninfected controls with gastrointestinal symptoms, and 178 uninfected healthy controls using indirect hemagglutination antibody assay. We assessed the rate of intestinal colonization by E histolytica and E dispar by fecal antigen and polymerase chain reaction tests in 332 asymptomatic HIV-infected persons and 144 of the 178 uninfected healthy controls. Results Forty-nine (5.2%) of 951 HIV-infected persons had 51 episodes of invasive amebiasis. A high indirect hemagglutination antibody titer was detected in 39 (6.2%) of 634 HIV-infected persons compared with 10 (2.3%) of 429 uninfected controls with gastrointestinal symptoms and 0 of 178 uninfected healthy controls (P<.001). Stool specimens from 40 (12.1%) of 332 HIV-infected persons and 2 (1.4%) of 144 uninfected healthy controls were positive for E histolytica or E dispar antigen (P<.001). Ten (25.0%) of the 40 antigen-positive stool specimens from HIV-infected persons contained E histolytica. Conclusion Persons infected with HIV in Taiwan are at increased risk for invasive amebiasis and exhibit a relatively high frequency of elevated antibody titers and intestinal colonization with E histolytica.

Archives of Medical Research (1)

Background
It is well documented that Giardia duodenalis undergoes surface antigenic variation both in vivo and in vitro. Proteins involved have been characterized and referred to as VSP (variable surface protein).

Methods
Two cloned cDNA inserts of 0.45 and 1.95 kb were obtained from G. duodenalis expression library and sequenced. Comparison sequence analyses were made against Genbank. PCR analysis was performed on G. duodenalis isolates to identify isolates bearing genes encoding such a peptide. Specific antiserum was prepared against 450-bp encoded peptide and tested by Western blot, immunofluorescence, and inhibition of adhesion of G. duodenalis to target cells.

Results
We cloned and characterized a G. duodenalis 450-bp DNA fragment; its DNA sequence analysis revealed that this fragment displayed 99% identity with vsp9B10A gene. Predicted amino acid sequence for this fragment also had significant (99%) identity to VSP9B10A. A second 1.95-kb insert, which encompassed the 450-bp cDNA fragment, was also isolated; its DNA and amino acid sequence displayed 99.5% identity with vsp9B10A gene and 99.2% with the corresponding inferred protein, respectively. This inferred protein contained 24 Cys-X-X-Cys motifs and long ORF of 642 amino acids. PCR analysis showed that DNA sequence encoding a fragment of this gene was present in P1, CIEA:0487:2-C-8 clone and in INP:180800-B2 G. duodenalis human isolates, while it was absent in sheep isolate of G. duodenalis INP:150593-J10.

Conclusions
Immunofluorescence analysis using antibodies raised against the peptide encoded by 450-bp fragment showed that expression of this epitope varies on trophozoite surface of the C-8 Mexican clone and is involved in parasite adhesion to target epithelial cells.

Archives of Oral Biology


Rat cystatin S and rat cystatin C are members of family 2 (cystatin) of the cystatin superfamily. All members of the cystatin family inhibit cysteine proteinases to varying degree. The expression of these two inhibitors, which have a 48% similarity at the nucleotide level, was studied in the submandibular gland using reverse transcriptase-polymerase chain reaction (RT-PCR), Northern blot hybridization and in situ hybridization with digoxigenin-labelled DNA probes. Both inhibitors were expressed in the serous acinar cells of the submandibular gland. In accord with previous findings, cystatin S mRNA was induced by the [beta]-adrenergic agonist isoproterenol. The level of cystatin S mRNA, which was very low in the glands of untreated rats and was demonstrable by RT-PCR but not by Northern blot hybridization, was not altered by acute inflammation produced by turpentine. Neither the administration of isoproterenol nor acute inflammation had any effect on the level of cystatin C mRNA, indicating that [beta]-adrenoceptors are not involved in the regulation of the cystatin C gene(s) in the submandibular gland. The data indicate that these two closely related genes, expressed in the same cells, are differently regulated. The consequence of this difference in gene regulation on the physiological and pathological roles of these inhibitors.
remains to be established.


SummaryApoptosis, also known as programmed cell death, is regulated by a number of inhibitory or stimulatory factors. In addition to the pro- and anti-apoptotic Bcl-2 family proteins, there is also a family of inhibitors of apoptosis protein (IAP). Survivin, a member of this IAP family, is selectively upregulated in most tumours. The objective of the present study was, therefore, to investigate the protein and mRNA expression of survivin, as well as the methylation status of the CpG sites in exon 1 of the survivin gene for 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal-pouch squamous-cell carcinomas. Immunohistochemical analysis for protein expression, RT-PCR for mRNA expression, and a PCR-based methylation assay were performed on 26 samples of hamster buccal pouches. The total study population was assigned into either one experimental group (15-week DMBA treatment; n = 13) or two control groups (untreated: n = 6; mineral-oil treated n = 7). Cytoplasmic staining of survivin protein and mRNA were detected in all of the hamster buccal-pouch tissue specimens treated with DMBA, whereas neither survivin protein nor survivin mRNA were noted for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens. Furthermore, all the untreated and mineral-oil treated samples had a survivin-methylated allele, whereas the DMBA-treated cancerous tissues showed no evidence of survivin methylation. The results suggest that survivin may play an important role in DMBA-induced hamster buccal-pouch carcinomas, and that the gene expression may be modulated by an epigenetic mechanism.


http://www.sciencedirect.com/science/article/B6T4J-4BWF993-1TG/2/b49a89ac26ac6e3fb41e88d1f9dd4b97

The fate of the progeny of human oral gingival keratinocytes was mapped in stratified epithelial tissues in vitro by following the expression of a marker gene in genetically related clones. Oral epithelial progenitor cells were genetically marked at high efficiency by transducing them with a retrovirus vector that carried the gene for a histochemically detectable product, Escherichia coli [beta]-galactosidase ([beta]-gal). These cells were then grown in submerged cultures and on collagen rafts at the air-liquid interface to demonstrate the distribution of genetically marked cells in a differentiating tissue in vitro. The dynamics of transduced cells showed that clonally related cells were arranged in discrete units of labelled cells and these clusters were defined as 'clonal proliferation units'. The size and configuration of these units were related to the proliferative potential and differentiating capacity of the cell that was initially transduced. This model demonstrates the relation between clonally related cells and tissue architecture for oral keratinocytes in vitro.

Retinoids alter the patterning of murine odontogenesis in vivo and stimulate epithelial proliferation. Because odontogenesis is dependent on proliferation of mandibular epithelium, the effects of retinol on the patterning of odontogenic epithelium were studied. These experiments control for developmental stage, applied retinoid concentration and duration of exposure. Explants exposed for 24 h to 0.1 μg/ml of retinol exhibited enhanced odontogenesis. Day-9 mandibles exposed to retinol at 1-5 μg/ml had altered epithelial patterns consistent with those in previous in vivo experiments, including supernumerary epithelial buds in regions associated with supernumerary incisors in vivo. These changes were associated with a dose-dependent increase in epithelial proliferation and a prolonged expression of epidermal growth factor (EGF) mRNA. Altered expression of EGF mRNA may be responsible for the disrupted pattern of the dental lamina. This is the first report of a retinoid-induced alteration in EGF mRNA expression.


Reverse transcription and cDNA amplification (polymerase chain reaction) of total RNA preparations were used to characterize the expression of EGF mRNA in the mandibular arch of day 9-17 mouse embryos. EGF mRNA was present in mandibles at day 9 and 10 but not at days 11-17. Separate RNA preparations from epithelium and mesenchyme at day 10 revealed EGF mRNA in both components.


Mycoplasma fermentans and other mycoplasma species may be associated with human immunodeficiency virus infection. Little is known about the ecology of this micro-organism and its natural habitat. A polymerase chain reaction (PCR)-based assay was used to detect M. fermentans in whole saliva. The hypothesis was tested that M. fermentans is present on the mucosal surfaces of the mouth and oropharynx. Whole saliva was collected from 110 adults. The 206-bp amplification product of DNA purified from these samples was detected in ethidium bromide-stained 6% polyacrylamide gels in 49 (44.5%) samples tested. All samples were confirmed by Southern blotting with a probe based on an internal sequence of the expected amplification product. The data suggest that this organism is often found in saliva and on oropharyngeal mucosal surfaces. Saliva may play a part in its transmission between individuals. Saliva sampling may be helpful in further studies of the ecology and distribution of the micro-organism in human populations.

Periodontal ligament (PDL) cells have osteoblast-like features and are capable of differentiating into osteogenic cells. As human osteoblasts express oestrogen receptor mRNA, it is possible that PDL cells do so also, but findings have been conflicting. To determine whether they do express oestrogen receptor mRNA, the reverse transcriptase-polymerase chain reaction was performed with two different primers. Cells were obtained from a healthy periodontal ligament of premolar extracted for orthodontic reasons. The human breast adenocarcinoma cell-line MCF7 was used as a positive control. Expression of oestrogen receptor mRNA was detected in PDL cells with one of the primers but with less intensity than in MCF7 cells. Southern hybridization confirmed these results. These findings suggest that PDL cells express oestrogen receptor mRNA at low levels.


A recent preliminary (unpublished) study showed that phosphodiesterase (PDE) 3A and 3B are expressed in rat submandibular glands. Here, PDE3 activity was detected in homogenates of rat submandibular gland acinar epithelial (SMIE) cells, but not rat A5 (epithelial duct) cells. Most of the PDE3 activity in SMIE cells was recovered in the particulate fraction. Only PDE3B mRNA was detected by reverse transcription-polymerase chain reaction in RNA from SMIE cells. The nucleotide sequence of the fragment was identical to the sequence of rat PDE3B. The PDE3 specific inhibitor, OPC3689 (10 and 50 [mu]M), inhibited the growth of SMIE cells (19 and 63%), but not A5 cells. As the submandibular gland contains many types of cells, these results indicate that PDE3B may regulate a cAMP pool that is important in submandibular gland acinar epithelial cell function.


SummaryChanges in intracellular Ca2+ concentration ([Ca2+]) induced by agonists were simultaneously monitored in rat submandibular acini and ducts using a Ca2+ imaging system. Substance P (SP) elicited marked increases in [Ca2+] in acini but not in ducts. Carbachol (CCh) increased [Ca2+] in both acini and ducts, but the maximal level was higher in acini than in ducts. In contrast, epinephrine (Epi) also induced an increase in [Ca2+] in acini and ducts, but to a greater extent in ducts than in acini. Isoproterenol (ISO) caused a small but significant increase in [Ca2+] in ducts but not acini. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using total RNA extracted from highly purified acinar and ductal cells showed that substance P receptor mRNA was present in acini at higher levels than in ducts. In contrast, [alpha]1a-adrenoceptor mRNA was more strongly expressed in ducts than in acini. The muscarinic receptors (M3 and M5) and [beta]-adrenoceptors ([beta]1 and [beta]2) were expressed at equivalent levels in both cell types. These results confirm that acini and ducts exhibit significant differences in agonist-induced Ca2+ responses. Furthermore, substance P- and epinephrine-induced Ca2+ responses were consistent with receptor mRNA expression in acini and ducts, but carbachol- and isoproterenol-induced [Ca2+] increases were not.
The expression of mRNA for amylase was examined using the reverse transcriptase-polymerase chain reaction (RT-PCR). An amylase product was strongly detected in parotid and pancreas, but less strongly in liver. The degree of identity between the PCR products was assessed by restriction-enzyme mapping using two restriction enzymes, EcoRI and Scal, and DNA sequencing. The PCR product from pancreas was cut by both EcoRI and Scal, while the products from parotid and liver were cut by EcoRI but not by Scal. The sequence of the parotid product was 90.4% homologous to that of the pancreas, and 100% homologous to that of the liver. These results indicate that the same amylase mRNA may be expressed in parotid and liver. In addition, the expression of amylase mRNAs in other rat tissues was investigated using RT-PCR, and the sensitivity of each PCR product to Scal was tested. A weak single band was detected in submandibular gland, sublingual gland and stomach. Scal digestion cut the stomach product into two fragments, but had no effect on the submandibular and sublingual products. Thus, it may be possible to classify amylase isoenzymes into pancreatic and parotid types based on the sensitivity of their PCR products to Scal.

The discriminative power of the arbitrarily primed polymerase chain reaction (AP-PCR) in differentiating between Streptococcus mutans and Strep. sobrinus species, serotypes and clones was investigated. Mutans streptococcal isolates (127) obtained from 65 individuals (1-10 isolates per individual) were AP-PCR typed separately with two random primers, OPA-05 and OPA-13. Bacterial cell lysates were used as a template in PCR reactions, which made AP-PCR easy and fast to perform. Eighty-one isolates from 19 individuals were also ribotyped to compare the discriminative ability of ribotyping and AP-PCR techniques. AP-PCR performed with the two primers differentiated between Strep. mutans and Strep. sobrinus isolates, but neither primer detected serotype-specific amplification products. OPA-05 distinguished two main AP-PCR patterns among Strep. mutans isolates and one main pattern among Strep. sobrinus isolates, whereas OPA-13 found one main AP-PCR pattern among Strep. mutans isolates and two main patterns among Strep. sobrinus isolates. Ribotyping and AP-PCR revealed 40 and 33 different types among 81 selected isolates, respectively. Both techniques detected intra-individual heterogeneity in 16 out of 19 participants. The results indicate that AP-PCR has good discriminative ability in differentiating between mutans streptococcal clones and that the technique is suitable for epidemiological studies on mutans streptococci.
Background: The exact pathomechanism of inflammation progress and fibrosis in chronic obstructive sialadenitis is unknown. The aim of the present study was to assess whether there is an association between transforming growth factor beta (TGF-[beta]) and fibrogenic process of chronic sialadenitis. Methods: Tissue samples of 12 patients with chronic sialadenitis and 4 normal tissue samples of the submandibular gland were examined immunohistochemically for identification of TGF-[beta]. TGF-[beta]1 messenger RNA (mRNA) expression was analysed semiquantitatively using reverse transcription polymerase chain reaction and gel electrophoresis to correlate its expression levels with stages of the disease. Results: TGF-[beta] positive cells could be found in the secretory duct system of all examined samples. However, an intense TGF-[beta] immunoreactivity was observed in inflamed salivary glands. With progress of disease TGF-[beta]1 mRNA expression increases significantly. Conclusion: Expression of TGF-[beta] in chronic sialadenitis and its apparent increase in advanced stages of the disease, suggests that this growth factor may play a role in glandular fibrosis.


http://www.sciencedirect.com/science/article/B6T4J-4135WX1-6/2/a368ff50b41f84ec854f0dcc2dec869f

Primary cultures of dental papilla-derived cells have a limited lifespan in vitro and can be maintained only up to passage 7-9 before showing senescence, but in vitro investigations often require a large number of cells showing phenotypic characteristics of the original tissue. To overcome this shortcoming, second-passage cells established from calf molar tooth germs by enzymatic pretreatment of the dental papilla were transfected by electroporation with pSV3neo, coding for the oncogene simian virus 40 large t antigen and a neomycin-resistance gene. Under selection by G418 (neomycin), four cell clones were isolated by single cell dilution at passage 15. Integration of simian virus 40 large t antigen and expression of the gene products were determined in cell clones by polymerase chain reaction (PCR) and immunohistochemistry. Four transfected cell lines (clones B, C, D and no. 12) were maintained in culture for over 1.5 years. For cell characterization, gene expression of procollagen [alpha]1 (I) and osteocalcin was evaluated by reverse transcriptase (RT)-PCR with cDNA obtained from the established cell lines at passage 20. Expression of collagen type I, osteocalcin and dentine phosphoprotein was evaluated immunohistochemically at passage 20 and after 1.5 years of continuous cell culture. Gene expression and the expression of mineralized tissue-specific proteins was demonstrated with RT-PCR and immunohistochemistry within all four immortalized cell lines. Expression of dentine phosphoprotein was observed in three simian virus 40 large t antigen-transfected cell lines, suggesting the immortalization of odontoblast-like cells in vitro. Thus, transfection of bovine dental papilla-derived cells resulted in immortal cell lines exhibiting phenotypic characteristics of the original tissue.


http://www.sciencedirect.com/science/article/B6T4J-3T4D5S4-6/2/12c2a5d507c888904b36b93d48caa59

With immunocytochemistry numerous nerve fibres containing neuropeptide Y (NPY) were found in human molar pulp tissue, often around small blood vessels. Reverse transcriptase-polymerase chain reaction, using specific primers, detected mRNA of the human NPY Y1 receptor in the human pulp tissue. Thus, both NPY-containing nerve fibres and NPY Y1 receptor mRNA are present in human tooth pulp, possibly regulating vascular tone and pain perception.
Numerous nerve fibres containing calcitonin gene-related peptide (CGRP) were found by immunocytochemistry in human molar pulp. These nerves were often seen around small blood vessels and as free endings without vascular contact. In the trigeminal ganglion a large number of CGRP-immunoreactive nerve-cell bodies, mostly of small to medium size, was encountered. Reverse transcriptase-polymerase chain reaction, using specific sense and antisense primers, detected mRNA expression of the human CGRP1 receptor in the pulp tissue and the trigeminal ganglion. Thus, both CGRP-containing nerve fibres and CGRP1 receptor mRNA are present in human tooth pulp, where they may be involved in the regulation of vascular tone and other local reactions to injury.

Cultured dental follicle cells from rat mandibular molars transcribe colony-stimulating factor-1 (CSF-1) mRNA as determined by reverse-transcription polymerase chain reaction. In turn, the CSF-1 mRNA appears to be translated, as seen by immunoperoxidase staining. Interleukin 1[alpha] (IL-1[alpha]) stimulates increased transcription of the CSF-1 gene in a concentration- and time-dependent manner. Moreover, CSF-1 itself has an autocrine effect on transcription of the CSF-1 gene. Because others have shown that in vivo injection of CSF-1 accelerates tooth eruption and because the dental follicle is required for eruption to occur, this study demonstrates the possible relation between CSF-1 and the follicle; namely, the source of CSF-1 for tooth eruption might be the dental follicle. In turn, regulation of gene expression for CSF-1 by IL-1[alpha] and CSF-1 may play a part in signalling the onset of tooth eruption.

Tooth eruption requires the presence of the dental follicle, a loose connective tissue sac that surrounds each unerupted tooth. The follicle appears to regulate many of the cellular and molecular events of eruption, including the formation of osteoclasts needed to resorb alveolar bone to form an eruption pathway. To that end, the expression of the tumour necrosis factor-[alpha] (TNF-[alpha]) gene was examined in the dental follicle as a possible regulator of osteoclastogenesis. TNF-[alpha] was expressed slightly in the dental follicle of the first mandibular molar of the rat beginning at day 3 postnatally, but maximal expression was seen at day 9, a time that correlates with a slight burst of osteoclast formation seen at day 10 postnatally. In vitro, TNF-[alpha] was not expressed constitutively in the follicle cells but incubating them with interleukin 1[alpha] resulted in a strong expression of TNF-[alpha] after only 0.5 h.
itself enhanced monocyte chemotactic protein 1 (MCP-1) and vascular endothelial growth factor (VEGF) gene expression. It also slightly decreased the expression of osteoprotegerin after 3-h incubation but this returned to the control level at 6 h. MCP-1 and VEGF could aid in recruiting mononuclear cells (osteoclast precursors) to the dental follicle. In addition to the potential role of TNF-[alpha] in tooth eruption, this study suggests that the periodontal ligament derived from the dental follicle might have the capacity to synthesize TNF-[alpha], and thereby contribute to the destructive events of periodontitis.


The prevalence and cellular distribution of human herpesvirus 7 (HHV-7) in archival labial salivary glands were analysed for virus-specific DNA sequences by polymerase chain reaction (PCR) and in situ hybridization signals. In addition, the cellular expression of HHV-7-encoded protein was detected by immunohistochemical staining with a virus-specific monoclonal antibody. Eleven of 20 samples were positive for the HHV-7 DNA sequence by PCR. Eighteen of 20 tissues analysed by in situ hybridization showed signals in ductal, serous and mucous cells. Some nuclei of these cells and also the myoepithelial population were positive. In immunolocalization studies, all 20 salivary glands consistently showed HHV-7-expressed protein in the cytoplasm of ductal cuboidal and columnar cells. The protein was also found in the cytoplasm of mucous and serous acinar cells that were immunopositive for HHV-7. The observations are consistent with the suggestion that the labial salivary gland is a site for virus replication, potential persistence and a source of infective HHV-7 in saliva.


The mouse is useful in studies of vascular biology because of its well-defined genetics and because the mouse genome can be manipulated. However, because only small amounts of mRNA can be extracted from blood vessels, the quantification of gene expression in individual mice is difficult. Endothelial NO synthase (eNOS) plays a major role in the regulation of vascular tone and growth. In addition, there appear to be sex differences in the production of NO under basal conditions in mouse aortas. The goals of this study were to develop a real-time polymerase chain reaction (PCR) method to quantify eNOS mRNA in blood vessels from mice and to examine eNOS mRNA levels in vessels from male and female mice. Blood vessels were isolated from C57BL/6 mice. Total RNA from individual mice was isolated and reverse-transcribed. The number of molecules of eNOS mRNA (after reverse transcription) was determined against cDNA standards, with 18S rRNA used as a control for RNA input and reverse-transcription efficiency. When expressed as copy numbers per nanogram of total RNA or as the ratio of eNOS to 18S
rRNA, eNOS mRNA was lower in the aortas of female mice than in those of male mice at 7 to 9 months of age. In contrast, no difference in eNOS mRNA was found in the aortas of 2-month-old mice. In addition, eNOS mRNA levels were similar in the carotid, cerebral, and coronary arteries. These findings provide the first quantitative measurements of eNOS mRNA by using real-time PCR in the vessels of mice and suggest age- and sex-related differences in the basal levels of eNOS mRNA in mice. In addition, the eNOS region that was used for real-time PCR was amplified and sequenced for monkeys and other species. With modifications, this region may be used to design real-time PCR for eNOS in other species.


http://atvb.ahajournals.org/cgi/content/abstract/24/7/1253

Objective-- Determining the role of specific muscarinic (M) receptor subtypes mediating responses to acetylcholine (ACh) has been limited by the specificity of pharmacological agents. Deletion of the gene for M5 receptors abolished response to ACh in cerebral blood vessels but did not affect dilation of coronary arteries. The goal of this study was to determine the M receptors mediating responses to ACh in coronary circulation using mice deficient in M2 or M3 receptors (M2-/-, M3-/-, respectively). Methods and Results-- Coronary arteries from respective wild-type, M2-/-, or M3-/- mice were isolated, cannulated, and pressurized. Diameter was measured with video microscopy. After preconstriction with U46619, ACh produced dose-dependent dilation of coronary arteries that was similar in wild-type and M2-/- mice. In contrast, dilation of coronary arteries from M3-/- mice to ACh was reduced by ≈80% compared with wild type. The residual response to ACh was atropine insensitive. Relaxation of coronary arteries to other stimuli was similar in M2-/- and M3-/- mice. Similar results were obtained in aorta rings. Conclusion-- These findings provide the first direct evidence that relaxation to ACh in coronary circulation is mediated predominantly by activation of M3 receptors. This study examined the M receptor subtype (M2 versus M3 receptors) involved in the response of coronary circulation to ACh using mice deficient in the genes for M2 and M3 receptors. M3 receptor activation and not M2 receptors primarily mediates responses to ACh in the coronary circulation.


http://atvb.ahajournals.org/cgi/content/abstract/22/5/805

Peroxisome proliferator activated receptor (PPAR) alpha is a member of the nuclear receptor superfamily that regulates key proteins involved in fatty acid oxidation, extracellular lipid metabolism, hemostasis, and inflammation. A L162V polymorphism at the PPARA locus has been associated with alterations in lipid and apolipoprotein concentrations. We studied the association among lipids, lipoproteins, and apolipoproteins and the presence of the L162V polymorphism in 2373 participants (1128 men and 1244 women) in the Framingham Offspring Study. The frequency of the less common allele (V162) was 0.069. The V162 allele was associated with increased serum concentrations of total and LDL cholesterol in men (P=0.0012 and P=0.0004, respectively) and apolipoprotein B in men (P=0.009) and women (P=0.03 after adjustment for age, body mass index, smoking, and use of {beta}-blockers, diuretics or estrogens). Apolipoprotein (apo) C-III concentrations were higher in carriers of the V162 allele. The association of the L162V polymorphism on LDL cholesterol concentration was greatest in those who also carried the E2 allele at the APOE locus and the G allele at the APOC3 3238C>G polymorphism. This suggests that alterations in triglyceride-rich lipoprotein metabolism may be
involved in the generation of the increase LDL cholesterol observed with the L162V PPARA polymorphism.

Atherosclerosis (31)


http://www.sciencedirect.com/science/article/B6T12-4B3DVRH-2/2/8a4977f407a4851e3d556d9bb3381375

Objective: Several studies show that the inflammatory component in atherosclerosis may contribute to increased risk for cardiovascular disease (CVD). Interleukin-6 (IL-6) is a key pro-inflammatory and immune-stimulatory cytokine of presumed importance for CVD and the metabolic syndrome. Methods and results: In this case-control study, 1179 surviving myocardial infarction (MI) cases and 1528 healthy controls were genotyped for three IL-6 promoter SNPs, and serum concentrations of IL-6 and C-reactive protein (CRP) were measured. In men, MI risk assessed as odds ratios (OR) was higher with increasing IL-6 levels, with the highest compared to the lowest IL-6 quartiles giving an OR of 2.7 [95% CI 1.7-4.4]. The ORs were independent from the effects of elevated CRP which were associated with modest MI risks (OR=1.6 [95% CI 1.0-2.5]). Also, synergistic interactions between high IL-6 levels and hypercholesterolaemia further increased MI risk estimates. The -174C allele was associated with lower serum-insulin levels among male controls but did not significantly influence MI risk or IL-6 levels. Conclusions: Elevated IL-6 levels are important risk markers for MI in men, the risk being further enhanced through synergistic interaction with hypercholesterolaemia. The data provide no clear evidence that polymorphisms in the IL-6 promoter region play a significant role in the pathogenesis of MI, and it remains to be further evaluated whether or not the -174C allele is of relevance for insulin resistance.


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Serum levels of Lp(a) lipoprotein are under genetic control and a high level is a risk factor for atherosclerotic disease. We have examined the aorta of LPA transgenic mice and their non-transgenic litter mates who had all been given a regular, not lipid fortified diet. When sacrificed, the animals had an average age of 66 weeks. Lipid lesions were observed in the aorta of 13 out of 18 LPA transgenic mice and in five out of 21 non-transgenic animals. The difference is statistically significant. We conclude that LPA transgenic mice develop lipid lesions in aorta more frequently than non-transgenic animals, even on a diet with a low fat content. LPA transgenic mice on a normal diet could be a useful animal model for the study of spontaneous human atherosclerosis, its treatment and prevention.
Hepatic lipase (HL) is an important determinant of high-density lipoprotein (HDL) concentrations. A common C-to-T substitution at position -514 of the promoter region of the HL gene has been shown to be associated with HL activity and HDL cholesterol (HDL-C) levels. The current study examines the influence of this polymorphism on both levels and serial changes of HDL-C from childhood to adulthood in a community-based sample of 707 white and 291 black unrelated individuals aged 4-38 years using a repeated measures analysis. The frequency of the -514T allele was lower in whites than in blacks (0.228 vs. 0.545, PP=0.003) in white males with values in the order of T/T>T/C>C/C. Although a similar trend was seen, the genotype effect was not significant in white females and blacks. Further, the slopes of the age trajectories of HDL-C were similar in three genotype groups in blacks and whites. A sex-genotype interaction effect (P=0.043) on longitudinal profiles of HDL-C levels was found in whites, but not in blacks. White males showed a stronger genotype effect (3.6 mg/dl, P=0.003) than white females (0.5 mg/dl, P=0.601). Thus, the -514T variant of the HL gene is consistently associated with higher levels of HDL-C longitudinally since childhood, but not with rate of change over time. These results suggest that the HL gene may play an important role in the regulation of HDL-C levels from childhood to adulthood, especially in white males.

Apolipoprotein D (APOD, gene; apoD, protein) is a plasma high-density lipoprotein (HDL)-associated glycoprotein, with a putative role in the cholesterol (CHOL) transport pathway. An apoD protein polymorphism has been previously reported by us. The cathodically shifted pattern seen on isoelectric focusing gels, controlled by the APOD*2 allele, was found to be unique to populations of African ancestry. To characterize the molecular basis of the protein polymorphism and to identify new mutations, we used a combination of SSCP, DHPLC and DNA sequencing techniques to screen the entire coding region of the APOD gene. We identified three distinct missense mutations, including Phe36Val, Tyr108Cys, and Thr158Lys with frequencies ranging from 2.1 to 2.8% in 722 African blacks from Nigeria. In addition, a common 8 bp deletion polymorphism was observed in intron 1 with a carrier frequency of 30.1%. The missense mutation, Thr158Lys correlated with the APOD*2 allele of the protein polymorphism. None of the 454 Caucasians screened for these polymorphisms showed any variation. We also determined the effect of these polymorphisms on plasma lipid levels in the African black population by generalized linear model (GLM). The Val36 allele was associated with significantly decreased HDL3-C (P=0.027) and apoA-I (P=0.030) levels among females. The Lys158 allele was associated with significantly increased Lp(a) (P=0.018) and triglyceride (P=0.017) levels, among females and males, respectively. In addition, males heterozygous for both intron 1 and codon 108 polymorphisms showed significantly increased HDL-C (P=0.011), HDL3-C (P=0.041), HDL2-C (P=0.009), apoA-I (P=0.005) and decreased LDL-C (P=0.025) levels. The results of our study show that the APOD gene harbors several polymorphisms, which are unique to African populations. Further study of these polymorphisms may help to characterize the role of apoD in lipid metabolism, and in cardiovascular disease among African populations.
Chronic Chlamydia pneumoniae infection and autoimmunity to heat shock protein 60 (Hsp60) have both been documented to be associated with atherosclerosis. Herein, we studied the effects of C. pneumoniae infection and a diet with a low-cholesterol supplement on the development of autoantibodies to mouse Hsp60 and early lipid lesions in the aortic valve of C57BL/6JBom mice. In addition, pulmonary infection was investigated. C57BL/6JBom mice were given one to three C. pneumoniae inoculations and fed either a regular diet or a diet enriched with 0.2% cholesterol. Autoantibody responses against mouse Hsp60 developed in both diet groups when the mice were infected with C. pneumoniae and in uninfected mice fed a cholesterol-enriched diet. C. pneumoniae infections increased subendothelial foam cell accumulation in mice on a 0.2% cholesterol-enriched diet (p = 0.022), without apparent hypercholesterolemia. These in vivo data suggest that autoantibodies against mouse Hsp60 develop as a consequence of cholesterol feeding and repeated C. pneumoniae infections. Further, infectious burden increased early lipid lesions in C57BL/6JBom mice fed a cholesterol-enriched diet.

Macrophage infiltration, inflammatory processes and oxidatively modified low density lipoprotein (LDL) are known contributing factors in the formation of the atherosclerotic plaque. To determine whether a direct link might exist between these factors, we examined the effect of oxidized LDL upon proinflammatory cytokine production in adherent human peripheral blood mononuclear leukocytes. Oxidized LDL, as well as a combination of cholesterol and 25-hydroxycholesterol, induced tumor necrosis factor-[alpha] (TNF[alpha]) and interleukin-1[beta] (IL-1[beta]) mRNA as measured by quantitative real time PCR, by a maximum of two- to fourfold following a 24-h incubation. Analysis of cell culture supernatants revealed a concomitant stimulation of TNF[alpha] and IL-1[beta] secreted protein as determined by ELISA. Treatment of human peripheral blood mononuclear leukocytes with oxidized LDL or the combination of cholesterol and 25-hydroxycholesterol caused activation of p38[alpha] as determined by the ability of immunoprecipitated p38 to phosphorylate an ATF-2 fusion protein, a surrogate substrate of p38[alpha]. VK-19911 (Pyridine, 4-[4-(4-fluorophenyl)-1-(4-piperidinyl)-1H-imidazol-5-yl]dihydrochloride), a specific inhibitor of p38[alpha], prevented the induction of TNF[alpha] and IL-1[beta] by oxidized LDL in a dose-dependent manner. Activated p38[alpha] is known to be involved in the stabilization of cyclooxygenase-2 mRNA in response to stimuli such as lipopolysaccharide; however, in the setting of oxidized LDL-induced p38[alpha] activation, COX-2 mRNA levels were not affected. Taken together, the data imply a potential role for p38[alpha] activation in lipid-associated inflammatory processes.
Atherosclerosis is associated with arterial deposition of low density lipoprotein (LDL) and lipoprotein(a), Lp(a). Both lipoproteins have been detected in atherosclerotic vessels; however, while LDL has been shown to be only blood-derived, it is not clear whether Lp(a) is also produced within the vessel wall. In the present investigation we studied gene expression of apo(a) and apoB in human blood vessels. Aorta, carotid arteries and liver specimens from 29 adult and pediatric autopsy cases were studied by RT-PCR and Southern blot analysis with primers and probes specific for apo(a), apoB and GAPDH (a control housekeeping gene). The mRNA of apo(a), but not apoB, was found within the vessel wall in both adult atherosclerotic arterial vessels and in pediatric non atherosclerotic vessels. Neither apo(a) nor apoB mRNA was detected in femoral veins. To verify the nature of the detected transcripts, we cloned the 162 base pair (bp) RT-PCR product derived from the arterial wall total RNA. Nucleotide sequencing revealed 100% homology with the apo(a) gene. Thus, while LDL in atherosclerotic arteries is exclusively blood-derived, the accumulation of Lp(a) within the artery may be due in part to in situ production of apo(a) within the vessel wall.

The fatty acid transport proteins (FATPs) have been implicated in facilitated cellular uptake of non-esterified fatty acids (NEFAs), thus having the potential to regulate local and systemic NEFA concentrations and metabolism. Hypothesising that genetic variation within the FATP genes may affect lipid metabolism, we investigated a G/A substitution at position 48 in intron 8 of the fatty acid transport-1 (FATP1) gene with respect to associations with fasting and post-prandial plasma lipid and lipoprotein variables in 628 healthy 50-year-old Swedish men and 426 Swedish women, aged 37-65 years. A subset of 105 men with the apoE3/E3 genotype underwent an oral fat tolerance test. Although fasting plasma TG concentrations were not different, male A/A individuals had significantly higher post-prandial TG concentrations and VLDL1 (Sf 60-40 apoB100)-to-VLDL2 (Sf 20-60 apoB100) ratio compared to male G/A and G/G individuals. A/A individuals apparently failed to suppress plasma NEFA concentrations during the oral fat tolerance test. Furthermore, fasting plasma concentrations of the largest, most buoyant LDL subfraction (LDL-I) were significantly lower in carriers of the A allele in the male cohort. Electromobility shift assays and reporter gene studies indicated that binding of nuclear factors and effect on transcriptional activity differ between the intron 8 alleles. These findings suggest that through regulation of NEFA trafficking, FATP1 might play a role in post-prandial lipid metabolism and development of cardiovascular disease.

Linkage and association of the apo AI-CIII-IV gene region to familial combined hyperlipidemia (FCHL) was reported previously, based on the presence of genetic variants in the apo CIII and
apo AI gene. No data were available yet on the contribution of the apo A-IV locus. Two DNA variants in exon 3 of the apo A-IV gene, A (Thr)347T (Ser) and [CTGT]3-4 were characterized by sequencing the coding region of the apo A-IV gene and were analyzed in our Dutch FCHL cohort (30 probands, 159 affected relative, 317 unaffected relatives and 218 spouses). The genotype frequency of the A347T variant was different in probands and spouses. In probands no 2/2 carriers were found, resulting in a significant decreased frequency of the 2-allele (P3-4 variant between the groups. Homozygous 4/4 carriers in spouses had a more favorable lipid profile (LDL-cholesterol and apo B, P347T with other markers in the gene cluster, and the absence of linkage disequilibrium with [CTGT]3-4 marker and the MspI-AI marker in the apo A-I promoter showed that these two apo A-IV variants reside on different haplotypes from the apo A-I and apo C-III markers. This was illustrated by extensive haplotype analysis. The present data on the contribution of DNA variants in the apo A-IV gene support our previous observations that the apo AI-CIII-AIV gene cluster has a complex genetic contribution to FCHL both by conferring susceptibility and protection.


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Familial combined hyperlipidemia (FCHL) is a frequent cause of premature coronary artery disease. Affected family members are characterized by different combinations of elevated cholesterol and/or triglyceride levels. A reduction in lipoprotein lipase (LPL) activity has been observed in a subgroup of FCHL patients. Recently, we have demonstrated an increased frequency of mutations in the LPL gene in Dutch FCHL patients compared to normolipidemic controls. In the present study, we have applied a pedigree-based maximum likelihood method to study the effect of LPL mutations on the phenotypic expression of FCHL in families. In 40 FCHL probandi, three different previously reported mutations in the LPL gene were identified resulting in amino acid changes, D9N, N291S, and S447X. The D9N mutation in exon 2 appeared to be in strong linkage disequilibrium with a T->G substitution at position -93 in the promoter region of the LPL gene. We present data that the -93T->G/D9N haplotype is associated with significantly higher levels of LDL and VLDL cholesterol, and VLDL triglycerides. Interestingly, the effect was only observed in male carriers. In line with our previous observations, these results further sustain that the LPL gene is a susceptibility gene for dyslipidemia which explains part of the variability in the phenotype observed among FCHL family members.


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Familial combined hyperlipidemia (FCHL) is a heritable lipid disorder characterized by multiple lipoprotein phenotypes within a single family. Previously, we have shown an increased incidence of mutations in the LPL gene which was associated with elevated levels of very low density lipoprotein (VLDL) and decreased levels of high density lipoprotein among the families studied. Now, we report the results of our study on the hepatic lipase gene. We found the HL V73M variant to be present in four FCHL families. By means of a pedigree-based maximum log-likelihood method we analyzed the effect of this variant on the lipid levels in these families.
Carriers of the HL V73M variant revealed significantly higher levels of total cholesterol (PP<0.01). These findings show that the HL V73M mutant explains another part of the variability in the phenotype observed among FCHL family members, compared with mutations in the LPL gene. Family analysis shows that in these FCHL families, carriers of mutations in the LPL or HL genes have an increased risk for FCHL compared with their non-carrier relatives.


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Atherosclerotic plaques contain a significant number of macrophage foam cells and are associated with an inflammatory state. Inflammation induces the secretion from monocytes and other cells of cytokines, reactive oxygen species, proteinases and proteinase inhibitors among many other molecular species. AAT is prominent among the serine proteinase inhibitors and is an important regulator of leukocyte elastase and proteinase-3. It has been shown that the stable AAT-proteinase complex can upregulate AAT biosynthesis, and we have shown that the shorter, carboxyl terminal peptide (C-36) resulting from proteinase cleavage of AAT polymerizes, and in its fibrillar form alters cellular metabolism. To test for a possible link between the inflammation-generated C-36 peptide and cellular processes associated with atherogenesis, we have studied the effects of the fibrillar form of this peptide at varying concentrations on human monocytes in culture. We have found that fibrillar C-36 at concentrations of greater than or equal to 5 [mu]mol/l in monocyte cultures for 24 h significantly increases LDL binding and uptake, upregulates LDL receptors, induces cytokine production and glutathione reductase activity, and upregulates AAT synthesis. The expression of CD36 protein, LDL Scavenger receptor, is also upregulated by fibrillar C-36 and native LDL in the presence of C-36-activated monocytes is more oxidized than with unactivated control monocytes. The majority of monocytes cultured for 24 h in the presence of C-36 fibrils were transformed morphologically into macrophages. These data establish a direct molecular link, mediated by C-36 peptide of AAT, between inflammation and the oxidation and accumulation of lipid in monocyte-derived macrophages. This may be important for an understanding of the events conducing to atherogenesis.


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Heterozygous familial hypercholesterolemia (FH) is one of the most common potentially fatal single-gene diseases leading to premature coronary artery disease, but the majority of heterozygous FH patients have not been diagnosed. FH is due to mutations in the gene coding for the low-density lipoprotein (LDL) receptor, and molecular genetic diagnosis may facilitate identification of more FH subjects. The Danish spectrum of 29 different mutations, five of which account for almost half of heterozygous FH, is intermediate between that of countries such as South Africa, where three mutations cause 95% of heterozygous FH in the Afrikaners, and Germany or England, where there are many more mutations. In clinical practice, a strategy for the genetic diagnosis of heterozygous FH, tailored to the mutational spectrum of patients likely to be seen at the particular hospital/region of the country, will be more efficient than screening of the whole LDL receptor gene by techniques such as single-strand conformation polymorphism (SSCP) analysis in every heterozygous FH candidate. In Aarhus, Denmark, we have chosen to
examine all heterozygous FH candidates for the five most common LDL receptor gene mutations (W23X, W66G, W556S, 313+1G->A, 1846-1G->A) and the apoB-3500 mutation by rapid restriction fragment analysis. Negative samples are examined for other mutations by SSCP analysis followed by DNA sequencing of the exon indicated by SSCP to contain a mutation. If no point mutation or small insertion/deletion is detected, Southern blot or Long PCR analysis is performed to look for the presence of large gene rearrangements. In conclusion, our data suggest that an efficient molecular diagnostic strategy depends on the composition of common and rare mutations in a population.


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HMG-CoA reductase inhibitor drugs or 'statins' have been shown to effectively reduce plasma total cholesterol (CHOL), CHOL associated with low-density-lipoprotein (LDL), and triglycerides (TG). In addition, slight elevations in HDL-CHOL are also typically observed. Poloxamer 407 (P-407), a nonionic surfactant, effectively elevates both plasma CHOL and especially TG in a dose-controlled fashion and results in formation of atherosclerotic lesions in the aortas of C57BL/6 mice without the requirement of dietary cholic acid [1 and 2]. The purpose of the present study was to assess whether a typical statin, namely atorvastatin (Lipitor(R)) would significantly reduce P-407-induced hypercholesterolemia and hypertriglyceridemia as well as cause regression of atherosclerotic lesions resulting from administration of P-407 to C57BL/6 mice. C57BL/6 mice in the present study were treated with either normal saline (C, controls), 0.5 g/kg of P-407 (P), or a high-fat, high-cholesterol, cholate-containing diet (HF) for 120 days. Mice in all groups were then equally and randomly divided and treated with either atorvastatin or saline for an additional 120 days. Beginning at Day 121 and using mice in groups P and HF as an example, one-fourth of the mice in each group received 20 mg/kg per day of atorvastatin with either concomitant HF feeding or P-407 administration ('progression' treatment groups), one-fourth received 20 mg/kg per day of atorvastatin following cessation of HF feeding or P-407 administration, one-fourth received saline (placebo) with either simultaneous HF feeding or P-407 administration ('progression' placebo groups), and one-fourth received saline (placebo) following cessation of HF feeding or P-407 administration. Total plasma CHOL was significantly (P<0.05) elevated compared to plasma TG of C mice. With discontinuation of P-407 administration, total plasma TG declined rapidly in P mice with atorvastatin-treated mice typically demonstrating lower plasma TG concentrations relative to saline-treated P mice. Aortas of mice treated with 20 mg/kg per day of atorvastatin in both groups P and HF, whether maintained on the HF-diet or treated with P-407 from Day 120 to 240 or whether each treatment was terminated at Day 120, revealed no presence of atherosclerotic lesions relative to saline-treated mice and were indistinguishable from aortas retrieved from C mice. Atorvastatin at a dose of 20 mg/kg per day not only significantly reduced the plasma CHOL and TG concentrations, but also resulted in regression of atherosclerotic lesions induced in C57BL/6 mice by administration of P-407 or ingestion of a HF-diet containing cholic acid.


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Oxidized low-density lipoprotein (oxLDL) exhibits many atherogenic effects, including the promotion of monocyte recruitment to the arterial endothelium and the induction of scavenger receptor expression. However, while atherosclerosis involves chronic inflammation within the arterial intima, it is unclear whether oxLDL alone provides a direct inflammatory stimulus for monocyte-macrophages. Furthermore, oxLDL is not a single, well-defined entity, but has structural and physical properties which vary according to the degree of oxidation. We tested the hypothesis that the biological effects of oxLDL will vary according to its degree of oxidation and that some species of oxLDL will have atherogenic properties, while other species may be responsible for its inflammatory activity. The atherogenic and inflammatory properties of LDL oxidized to predetermined degrees (mild, moderate and extensive oxidation) were investigated in a single system using human monocye-derived macrophages. Expression of CD36 mRNA was up-regulated by mildly- and moderately-oxLDL, but not highly-oxLDL. The expression of the transcription factor, proliferator-activated receptor-[gamma] (PPAR[gamma]), which has been proposed to positively regulate the expression of CD36, was increased to the greatest degree by highly-oxLDL. However, the DNA binding activity of PPAR[gamma] was increased only by mildly- and moderately-oxLDL. None of the oxLDL species appeared to be pro-inflammatory towards monocytes, either directly or indirectly through mediators derived from lymphocytes, regardless of the degree of oxidation.


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Objective: In the past decade, elevated homocysteine concentration has achieved widespread recognition as an independent risk factor in the development of atherosclerosis. 3-Deazaadenosine (c3Ado) is a potent inhibitor and substrate for S-adenosylhomocysteine hydrolase and therefore may reduce homocysteine concentrations. The current study investigated the effect of c3Ado on serum homocysteine, atherosclerotic lesions, and the expression of adhesion molecules in apoE-knockout mice. Methods and results: Animals were placed on an atherogenic diet with or without c3Ado for 12 and 24 weeks. Frozen cross-sections of the aortic sinus and the proximal aorta were analyzed by computer-aided planimetry for fatty plaque formation. Macrophages, VCAM-1 and ICAM-1 were quantified by immunohistochemistry and oligo-cell reverse transcription polymerase chain reaction after laser microdissection. Application of c3Ado resulted in significant reduction of homocysteine levels by 35.9 and 45.3% after 12 and 24 weeks, respectively (P<0.05). Conclusion: Our results demonstrate that c3Ado induces a marked reduction of homocysteine concentrations which might explain in part the anti-atherogenic effect of the drug.


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Hyperhomocysteinemia is an independent risk factor of cardiovascular disease and associated with insulin resistance, although their causal relationship remains unclear. A previous report has shown that high concentration of homocysteine damages mitochondrial gene expression, function and structure. As we found recently, the mitochondrial DNA (mtDNA) contents are inversely correlated with insulin resistance parameters. Thus there is possibility that plasma total homocysteine (tHcy) level is somewhat correlated with mtDNA content. Sixty healthy women
(mean age 40.3±20.9 yr, range 18-78 yr) were recruited to investigate the correlation of plasma tHcy level and mtDNA content in peripheral blood. A significant negative correlation was found between plasma tHcy levels and mtDNA content (r=-0.507, Pr=0.407), total cholesterol (r=0.338), LDL-cholesterol (r=0.317) and insulin resistance (HOMA-IR score) (r=0.261); and a negative correlation with folate (r=-0.273). MtDNA content showed negative correlations with age (r=-0.407), BMI (r=-0.440), W/H ratio (r=-0.659), SBP (r=-0.350), total cholesterol (r=-0.340), triglyceride (r=-0.376), LDL-cholesterol (r=-0.349), fasting plasma insulin (r=-0.483), and insulin resistance (HOMA-IR score) (r=-0.423); and a positive correlation with folate (r=0.299). In this study, there was a significant inverse correlation between plasma tHcy level and mtDNA content. Further study will be warranted to elucidate the mechanism by which two factors are associated.


Background: An insertion/deletion (I/D) polymorphism in the gene encoding angiotensin-converting enzyme (ACE) has been associated with serum ACE levels. The association between the ACE I/D polymorphism and coronary heart disease is unclear. Electron-beam-computed tomography (EBT) is a technique to non-invasively quantify the amount of coronary calcification. We investigated the association between the ACE I/D polymorphism and coronary calcification. Methods and results: The Rotterdam Coronary Calcification Study is a population-based study in subjects aged 55 years and over. EBT scanning was performed in 2013 participants. Coronary calcification was quantified according to the Agatston score. The ACE I/D polymorphism was available for 1976 subjects. Geometric mean calcium scores in men with the II, ID and DD genotype were 167, 207 and 219, respectively. However, the difference in calcium score (p = 0.19 for ID versus II; p = 0.15 for DD versus II) and the trend (ptrend = 0.17) were not significant. Calcium scores in women with the II, ID and DD genotype were 44, 42 and 36, respectively. There were no significant differences in calcium score (p = 0.78 for ID versus II; p = 0.29 for DD versus II), neither was the trend (ptrend = 0.27). After we stratified on cardiovascular risk factors, no associations were present. Conclusion: The present study failed to show an association between the ACE I/D polymorphism and coronary calcification in the general population. Also, no significant associations were present between the ACE I/D polymorphism and coronary calcification in strata of cardiovascular risk factors.


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Matrix metalloproteinase 3 (MMP3) is expressed in human coronary atherosclerotic lesions and is known to be involved in degradation of the plaque and to be co-localized with calcium and fibrin deposits in advanced lesions, indicating a possible role of MMP3 in arterial calcification. The MMP3 gene promoter polymorphism leads to low promoter activity 6A6A, intermediate promoter activity 5A6A and high promoter activity 5A5A genotypes. To determine whether these genotypes predict the extent of atherosclerosis we investigated their association with different types of coronary lesions in an autopsy series of 300 middle-aged white Finnish men (aged 35-69 years) from the Helsinki Sudden Death Study (HSDS). Areas of the coronary wall covered with different atherosclerotic lesions were measured and MMP3 genotypes were determined by PCR and minisequencing. In men >=53 years the mean area of calcified lesion in the most severely affected coronary artery was significantly associated with the MMP3 genotype (P=0.029). Subjects with high promoter activity genotypes had on average larger calcified lesion areas than those with the low-activity genotype. The MMP3 genotype (P=0.025) persisted as an independent predictor of mean calcified lesion area after stepwise adjustment for age, BMI, hypertension, diabetes, number of affected vessels and smoking. These data provide evidence that the proposed effect of MMP3 in the process of atherogenesis may be modified by the MMP3 genotype.
We attempted to detect chlamydial antigens in canine atherosclerotic lesions from seven dogs by immunohistochemical technique using anti-Chlamydia psittaci (C. psittaci) polyclonal and anti-C. pneumoniae monoclonal antibodies. Immunopositive signals to both antibodies were recognized in the atherosclerotic lesions of the aortas, coronary and splenic arteries of all dogs. Positive signals were found in the foamy cytoplasm of infiltrated macrophages and extracellular matrices in the lesions. In some lesions, cytoplasm of the endothelial cells and smooth muscle cells was also immunopositive against both antibodies. By electron microscopy, chlamydial microorganisms were found in the cytoplasm of endothelial cells and smooth muscle cells. Using polymerase chain reaction (PCR), detection of C. pneumoniae DNAs were performed in the spleen, heart (coronary arteries) and kidney in one of the seven dogs. Positive 314 bp PCR products were obtained in all samples of the dog. These results confirmed the presence of viable Chlamydiae in atheromas and supported the conclusion that the organism may be an active factor in the pathogenesis of canine, as well as human atherosclerosis.


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Objectives:Macrophage migration inhibitory factor 1[alpha] (MIF), a cytokine with immunoregulatory functions has been suggested to be involved in atherosclerotic plaque development. However, little is known about MIF-inducing conditions in the atherosclerotic process and the association of MIF with plaque instability.Methods and results:Forty-two carotid endatherectomy samples from 36 patients and 4 aortic samples from young accident victims (as healthy controls) were analyzed for MIF staining. MIF expressing tissues in the atherosclerotic plaques are mainly mononuclear cells (MNCs), but also endothelial cells of intimal microvessels (MVECs). The magnitude and the intensity of their MIF expression was associated with the progression of plaques from early lesions (Stary I-III) to complicated plaque stages (Stary IV-VIII). In highly inflammatory and neovascularized regions of the plaques the colocalization of MIF expressing MNCs with CD40-L+ and angiotensin II (Ang II)-producing MNCs could be established. This finding supports the notion that CD40-L fusion protein and Ang II are able to induce MIF production in the monocytic cell line THP-1. Furthermore hypoxia ([less-than or equal to]1% O2) as a further proinflammatory and especially proangiogenetic factor was able to stimulate MIF secretion by THP-1, human monocytes and HUVECs. Hyperglycemia and insulin remained without effect.Conclusion:MIF is expressed in advanced atherosclerotic lesions in close correlation with signs of instability, such as mononuclear cell inflammation and neointimal microvessel formation. Furthermore, the colocalization of MIF with Ang II-producing MNCs and CD40-L+ cells in these plaques and the finding that proathero- and -angiogenic mediators such as CD40-L, Ang II and hypoxia are able to stimulate MIF expression in vitro suggest an important role of MIF in the modulation of atherosclerotic plaque stability.


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Chlamydia pneumoniae infection generally starts in the respiratory tract and probably disseminates systemically in the blood stream within alveolar macrophages. We investigated the
prevalence of C. pneumoniae DNA in peripheral blood mononuclear cells (PBMC) in patients with acute ischaemic heart disease. Samples of blood were obtained from 93 consecutive patients with acute ischaemic heart disease and from 42 healthy subjects, for detection of C. pneumoniae DNA in PBMC by polymerase chain reaction (PCR) and for serology. C. pneumoniae DNA in PBMC was detected in 25.8% (24/93) of the patients with acute ischaemic heart disease and in 4.8% (2/42) of the healthy subjects (P=0.008). C. pneumoniae IgG was found in 76.3% of patients and in 45.2% of healthy subjects (P=0.0008) while C. pneumoniae IgA was found in 59.1% and in 33.3%, respectively (P=0.01). No correlation was found between anti-C. pneumoniae antibody titers and positive PCR results. The detection of C. pneumoniae DNA in PBMC may aid in selecting patients who may benefit from antibiotic treatment; however, to support this contention, longitudinal studies on patients treated with antibiotics would also be necessary.


http://www.sciencedirect.com/science/article/B6T12-4D5KT9K-1/2/bba14efc20409ef97308d64fc6969177

Paraoxanase (PON 1), a high-density lipoprotein-associated enzyme, exerts an antiatherogenic effect by protecting low-density lipoproteins (LDL) against oxidation. A common polymorphism at codon 192(Q/R) of the PON 1 gene has been shown to be associated with an adverse lipoprotein profile and increased coronary artery disease (CAD) risk. However, these observations are based mostly on case-control studies involving relatively older adults. This study examined the frequency and phenotypic (lipoprotein variables) effect of the Q192R variant in a community-based sample of 1786 black and white young adults (mean age: 32.5 years; 69% white, 44% males). In addition, the genotypic effect of this polymorphism on ultrasonographically measured carotid artery intima-media thickness (IMT), a surrogate measure of CAD risk, was examined in a subsample of 436 young adults (mean age: 32.6 years; 70% white, 42% male). The frequency of the variant allele (R192) was higher in blacks than in whites (0.668 versus 0.297, P P = 0.041), whereas the opposite was true in blacks (P = 0.008). Neither the Q nor the R allele was associated with LDL cholesterol and triglycerides in both races. The genotypic effect on the carotid IMT adjusted for the covariates including lipoprotein variables was not apparent in blacks or blacks. However, among whites, the carotid IMT was lower in carriers (QR + R) versus non-carriers (QQ) of the variant allele among females (P = 0.008) and non-smokers (P = 0.026). In addition, the variant allele negated the adverse positive relationship between the carotid IMT and triglycerides among whites (P = 0.212 for carriers versus P < 0.001 for non-carriers). These results indicate a differential effect of the Q192R variant on HDL cholesterol in whites versus blacks and a beneficial interaction effect of the variant allele with individual's sex, smoking status or triglyceride levels on the carotid IMT among whites.


http://www.sciencedirect.com/science/article/B6T12-4806D7K-2/2/797b256e6671f8b4ab3db8864f8a2ec

The cholesteryl ester transfer protein (CETP) gene has been implicated in the variation of HDL levels but most studies have focused on only one or a few genetic variations. In order to properly understand the role of CETP in determining phenotype, it is necessary to examine the entire gene and all its common polymorphisms. The coding regions, adjacent introns, and proximal 5' and 3'
regions were resequenced from an ethnically diverse population. Novel and previously known polymorphisms were then characterized and associations with HDL and CETP mass levels determined. The polymorphism most highly associated with CETP was 629 bp upstream of the transcription start site while the polymorphism most highly associated with HDL was a VNTR 1946 bp upstream of the transcription start site. Genetic variation in the CETP gene is associated with protective HDL levels. The ethnic diversity of some SNPs and complex interplay among them dictate careful analysis of the whole gene prior to conclusions about the role of individual polymorphisms.


http://www.sciencedirect.com/science/article/B6T12-3VXJKB4-8/2/c523094d13494c1dc6f03495e52cb395

We compared biochemical and molecular methods for the identification of heterozygous carriers of mutations in the cystathionine [beta]-synthase (CBS) gene. Eleven relatives of seven unrelated patients with homocystinuria due to homozygous CBS deficiency and controls were studied with respect to total homocysteine concentrations before and after methionine loading. In addition, we determined CBS activity in cultured skin fibroblasts and tested for the presence of five known mutations by a PCR-based method in these seven patients, their relatives and controls. The results demonstrate that measurement of homocysteine after methionine loading and assay of CBS enzyme activity in cultured fibroblasts identify most but not all heterozygotes. There was significant correlation between homocysteine concentrations and CBS activities only after methionine loading ($r = 0.12, 0.48, 0.48$ and $0.50$ at 0, 4, 6 and 8 h, respectively). Among the homozygous patients, molecular approaches identified five T833C and two G919A mutations out of 14 independent alleles, confirming the studies of others that these represent the two most prevalent mutations. In addition, we found that three of six heterozygotes with the T833C allele had post-methionine loading homocysteine levels which overlapped with controls and of the other three, one (as well as an obligate heterozygote who did not carry any of the five mutant alleles tested) had CBS activity comparable to that of controls. These findings demonstrate that genotyping is useful as an adjunctive method for the diagnosis of the heterozygous carrier state of CBS deficiency.


http://www.sciencedirect.com/science/article/B6T12-435MFHN-X/2/1f952e6758459ba39b1a831bbeb27430

Platelets are thought to contribute to development of restenosis following percutaneous coronary interventions. The glycoprotein Ia/IIa complex is a major platelet collagen receptor, its surface expression being influenced by two, linked single nucleotide polymorphisms (C807T and G873A) in the glycoprotein Ia gene. T807 is associated with increased expression of this integrin receptor. We assessed whether T807 is associated with an increased risk of restenosis in 1769 consecutive patients treated with coronary stenting. 6-month follow-up angiograms were available in 82.4% of the patients. C807T genotype distribution was CC in 35.8%, CT in 47.6% and TT in 16.6% of the patients. Restenosis (diameter stenosis [ges]50% at follow-up angiography) occurred in 32.9% of CC, 31.5% of CT and 32.1% of TT patients ($P = 0.87$). The rate of major adverse cardiac events (death, myocardial infarction or need of reintervention) within 1 yr was 21.6% for CC, 21.7% for CT and 21.2% for TT patients ($P = 0.98$). Thus, carriage of the GP Ia T807 allelle is not associated with an increased risk of restenosis or unfavorable late outcome.
following coronary artery stenting.


http://www.sciencedirect.com/science/article/B6T12-45HD82P-3/2/2974f8d9ad0d3d1cd2c02504609e968

Low density lipoprotein receptor deficient (LDLR-KO) and apolipoprotein E deficient (apo E-KO) mice both develop hyperlipidemia and atherosclerosis by different mechanisms. The aim of the present study was to compare the effects of simvastatin on cholesterol levels, endothelial dysfunction, and aortic lesions in these two models of experimental atherosclerosis. Male LDLR-KO mice fed a high cholesterol (HC; 1%) diet developed atherosclerosis at 8 months of age with hypercholesterolemia. The addition of simvastatin (300 mg/kg daily) to the HC diet for 2 more months lowered total cholesterol levels by ~57% and reduced aortic plaque area by ~15% compared with the LDLR-KO mice continued on HC diet alone, PP<0.05. In contrast, in age-matched male apo E-KO mice fed a normal diet, the same treatment of simvastatin elevated serum total cholesterol by ~35%, increased aortic plaque area by ~15%, and had no effect on endothelial function. These results suggest that the therapeutic effects of simvastatin may depend on the presence of a functional apolipoprotein E.


http://www.sciencedirect.com/science/article/B6T12-3SY2X53-13/2/6d4720c6e4f07d139a8ea74b97b8c27

Tranilast (N(3,4-dimethoxycinnamoyl)anthranilic acid), an agent which in cell culture inhibits transforming growth factor-[beta] (TGF-[beta]) secretion and antagonises the effects of TGF-[beta] and platelet-derived growth factor (PDGF) on cell migration and proliferation, has been reported to reduce the incidence of restenosis after angioplasty in angiographically validated human clinical trials. We investigated in a rat model of balloon angioplasty whether tranilast's effects in vivo could be attributed to inhibition of expression of TGF-[beta] and/or its receptor types. Using a standardised reverse transcriptase-polymerase chain reaction (RT-PCR) assay, we examined the effects of three doses of tranilast (25, 50 and 100 mg/kg) on the expression of two TGF-[beta] isoforms, the types I and II TGF-[beta] receptors and two putative TGF-[beta] responses, induction of integrins [alpha]v and [beta]3 mRNA, 2 h after oral administration and 26 h after vessel injury. Tranilast attenuated in a dose-dependent and reversible manner the injury-induced increases in mRNA levels encoding TGF-[beta]1, TGF-[beta]3, two type I TGF-[beta] receptors ALK-5 and ALK-2, and the type II receptor T[beta]RII. At the highest dose mRNA levels encoding TGF-[beta]1 and T[beta]RII were attenuated to levels approaching or below those observed in uninjured vessels. Messenger RNAs encoding TGF-[beta]3, ALK-5 and ALK-2 were all attenuated by between 70 and 74% (all P[alpha]v and [beta]3 observed after vessel injury, by 90 and 72%, respectively. We also investigated, in cultured smooth muscle cells derived from injured carotid arteries, the extent to which tranilast (300 mg/l) attenuated any increases in expression of type I and type II receptors stimulated by PDGF-BB and TGF-[beta]1, growth factors implicated in smooth muscle cell migration and proliferation in injured vessels. Increases in mRNA levels of the type I receptors ALK-5 and ALK-2 induced by PDGF-BB and TGF-[beta]1 were almost completely prevented by tranilast. Tranilast also prevented the PDGF-BB induced increases in T[beta]RII but only partially inhibited the TGF-[beta]1 induced upregulation of T[beta]RII. We conclude that tranilast can inhibit transcriptional mechanisms associated with the upregulation of TGF-[beta] and its receptor types in balloon catheter injured vessels. It is possible
that these mechanisms contribute to its ability to reduce the frequency of restenosis after angioplasty.


http://www.sciencedirect.com/science/article/B6T12-4991WFK-5/2/7c2b35c72bcbf84b0bdad0b61b767772

Inflammation and innate immunity may play a role in the pathogenesis of atherosclerosis. The Asp299Gly polymorphism in the toll-like receptor 4 (TLR4) gene reduces responsiveness to lipopolysaccharide and has been associated with reduced incidence and slower progression of carotid atherosclerosis. We analyzed this polymorphism in relation to susceptibility to and severity of coronary artery disease. Methods: 1400 participants (mean age: 63 years, 31% female) in the Southampton Atherosclerosis Study were genotyped for the TLR4 Asp299Gly polymorphism using the tetra-primer PCR method. Results: There was no significant difference between the frequencies of the Asp/Gly or Gly/Gly genotypes combined, compared to the Asp/Asp genotype, in patients with 0, 1, 2 or 3 coronary arteries with >50% stenosis (\(\chi^2d.f.2=0.4, P=0.94\)). No associations were observed between genotype groups and cardiac risk factors (\(P>0.05\)). Conclusion: The findings of this study do not support the hypothesis that the TLR4 Asp299Gly polymorphism influences predisposition to and progression of coronary artery disease.

Auris Nasus Larynx  (1)


http://www.sciencedirect.com/science/article/B6T4M-45XTF6B-1/2/f6e4aebde90db7dc7530d069e607ad46

Objectives: Mutations in connexin26 (GJB2) are one of the most frequent causes of prelingual hearing impairment. Several different types of one-base deletions in exon2 were the most common type of GJB2 mutation regardless of ethnicity, including 35delG in American-European populations, 235delC in Japanese population and 167delT in Ashkenazi Jewish population. Various types of one-base substitutions were also considered to be causative mutations of GJB2 associated hearing impairment. This article describes a rapid and high-throughput screening procedure for the detection of one-base deletion/substitution in GJB2 with less invasive sampling procedure in the implication for the clinical application. Methods: 53 hearing-impaired children and 50 healthy controls were admitted to take part in this study program. DNA samples obtained from buccal swab were used to amplify the exon2 of GJB2, and single run with an automated sequencer was used to identify the one-base deletion. Single-base substitutions were also screened by primer-extension procedure with dye terminators. The presence of both types of mutations was confirmed by direct sequence of the GJB2 exon2. Results: Two of 50 controls (4%) included one-base deletion in GJB2 as heterozygote. 14 of 53 hearing impaired cases (26.4%) contained deletion in GJB2 either as homozygote (five cases) or heterozygote (nine cases) form. Sequencing analysis of whole exon2 of GJB2 identified all these deletions as
235delC. Primer-extension analysis revealed additional mutations with single base substitutions in three cases with compound heterozygote with 235delC. Conclusions: Rapid screening procedure of GJB2 can be potentially useful for the identification of prelingual deafness.

Autonomic Neuroscience (3)


http://www.sciencedirect.com/science/article/B6VT5-4CVCCXD-1/2/94e528ac378fb76d794f27bf5f99a7

Stimulation of nicotinic acetylcholine receptors (nAChR) excites peripheral sensory nerve fibres, but also exert antinociceptive effects. The differences in these nAChR-mediated effects could be related to the expression of different nAChR subtypes located on nociceptive neurons. In the present study, we focused on the recently described [alpha]10-nAChR subunit, and on [alpha]4 and [alpha]7 subunits, which are the most abundant subunits in the central nervous system. In nociceptive neurons from thoracic and lumbar dorsal root ganglia (DRG), nAChR subunits were found at transcriptional (RT-PCR), translational (immunohistochemistry) and functional levels. Cultured DRG neurons express mRNA for the subunits [alpha]2-7 and [alpha]10. The [alpha]-subunit proteins 4, 7 and 10 were colocalised in virtually all nociceptive neurons that were identified by immunoreactivity for the vanilloid receptor TRPV-1. These findings were corroborated by current recordings and calcium measurements, which revealed excitatory inward currents and calcium responses in capsaicin sensitive neurons.


http://www.sciencedirect.com/science/article/B6VT5-41WBD7V-1/2/65020520773ee4851ea39c7d8cd2af31

In studies of the central and peripheral autonomic nervous system, it has become increasingly important to be able to investigate mRNA expression patterns within specific neuronal populations. Traditionally, the identification of mRNA species in discrete populations of cells has relied upon in situ hybridization. An alternative, relatively simple procedure is 'multiplex' reverse transcription-polymerase chain reaction (RT-PCR), conducted on single neurons after their in vitro isolation. Multiplex single-cell RT-PCR can be used to examine the expression of multiple genes within individual cells, and can be combined with electrophysiological, pharmacological and anatomical (retrograde labelling) studies. This review focuses on a number of key aspects of this approach, methodology, and both the advantages and the limitations of the technique. We also provide specific examples of work performed in our laboratory, examining the expression of alpha2-adrenergic receptors in catecholaminergic cells of the rat brainstem and adrenal medulla. The application of single-cell RT-PCR to future studies of the autonomic nervous system will hopefully provide information on how physiological and pathological conditions affect gene expression in autonomic neurones.

http://www.sciencedirect.com/science/article/B6VT5-42RDTCG-3/2/753a0cda7b4b1f69fd24209566b53a61

The aim of this study was to investigate the expression of the [alpha]2-adrenergic receptors in the adrenal medulla, and to examine the mechanism by which clonidine and related drugs inhibit acetylcholine (ACh)-induced whole-cell currents in adrenal chromaffin cells. Reverse transcription-polymerase chain reaction (RT-PCR) performed on punches of rat adrenal medulla demonstrated expression of mRNA for the [alpha]2A-, [alpha]2B- and [alpha]2C-adrenergic receptors. Similar experiments conducted with tissue punches obtained from the adrenal cortex did not reveal expression of these receptor subtypes. Whole-cell currents were recorded in isolated chromaffin cells using the perforated-patch configuration. ACh (50 [mu]M) evoked inward currents with a peak amplitude of 117.8+/-9.3 pA (n=45; Vhol=-60 mV). The currents were inhibited in a dose-dependent manner (0.5-50 [mu]M) by clonidine, UK 14,304 and rilmenidine (agonists of [alpha]2/imidazoline receptors), as well as by SKF 86466 and efaroxan (antagonists). Adrenaline and noradrenaline (50-100 [mu]M) had no significant effect. Thus, although the adrenal medulla expresses mRNA for the [alpha]2-adrenergic receptors, the lack of agonist-antagonist specificity observed in our whole-cell recordings (in the absence of intracellular dialysis) provides additional evidence against the possibility that these inhibitory effects are mediated by classical [alpha]2 or imidazoline receptor interactions.

Behav. Ecol. (2)


http://beheco.oupjournals.org/cgi/content/abstract/13/5/670

One factor hypothesized to influence the reproductive behavior of individuals is the degree to which reproductive efforts are synchronized with others in the population. We asked whether the timing of a pair’s breeding cycle, relative to cycles of pairs on neighboring territories, affected rates of extrapair mating over 2 years in a Wyoming population of house wrens (Troglodytes aedon). Extrapair young (identified using 5 microsatellite loci) occurred in 74% of nests of 19 pairs whose cycles began later than cycles of one or more neighbors compared to only 26% of nests of 27 pairs whose cycles began earlier than, or simultaneously with, cycles of all neighbors. Extrapair offspring occurred in 65% of 17 nests belonging to males who initially settled and began nesting early relative to neighbors but who were forced to renest late after we removed their first mates. Rates of cuckoldry were not significantly different for forced-late and naturally late males. Our experimental approach controlled for possible effects of male quality, clearly demonstrating an effect of timing of breeding on extrapair mating activity.

Although orang-utans live solitary lives most of the time, they have a complex social structure and are characterized by extreme sexual dimorphism. However, whereas some adult male orang-utans develop full secondary sexual characteristics, such as cheek flanges, others may stay in an "arrested" unflanged condition for up to 20 years after reaching sexual maturity. The result is a marked bimaturism among adult males. Flanged males allow females to overlap with their home range and often tolerate the presence of unflanged males. However, wherever possible flanged males actively prevent unflanged males from copulating with females. Two competing hypotheses, previously untested, have been advanced to explain male reproductive behavior and bimaturism in orang-utans: (1) the "range-guardian" hypothesis, which asserts that the flanged males are postreproductive and defend a range in which they tolerate sexually active, unflanged male relatives; and (2) the "female choice" hypothesis, which asserts that flanged males tolerate unflanged males in their range because they rely on female preference to favor flanged males. We investigated these hypotheses and a third hypothesis that the two male morphs represent co-existing alternative male reproductive strategies ("sitting, calling, and waiting" for flanged males versus "going, searching, and finding" for unflanged males). Fecal samples were collected from a well-studied population in Indonesia, and eight human microsatellites were analyzed for 30 individuals that have been behaviorally monitored for up to 27 years. By carrying out paternity analysis on 11 offspring born over 15 years, we found that unflanged males fathered about half (6) of the offspring. Relatedness between successful unflanged males and resident dominant males was significantly lower than 0.5, and for some unflanged/flanged male pairs, relatedness values were negative, indicating that unflanged males are not offspring of the flanged males.


A number of studies suggest melanocortin (MC) system involvement in nociceptive modulation. Although the mechanism through which this occurs is still unknown, experimental evidence would suggest a primary role of MC4 receptors. To further investigate the implication of this MC receptor subtype in chronic pain, we have studied the effects of several MC antagonists on spinal nerve ligation-induced nociceptive behavior in rats. The intrathecal injection of synthetic antagonists with different selectivity to MC4 receptor and of an endogenous antagonist (Agouti related protein; AgRP) reduced mechanical allodynia in neuropathic rats, as measured by von Frey hair test. Treatments produced an anti-allodynic effect at the dose of 1.5 nmol (25-30% maximum possible effect, MPE, P < 0.05). To further investigate the possible physiological role of AgRP in pain modulation we studied its expression in both sham and neuropathic rat spinal cord and dorsal root ganglia (DRG) by quantitative real time PCR and immunohistochemistry. AgRP was present in both spinal cord and DRG, and its expression, was unchanged in neuropathic animals. In conclusion MC4 receptor antagonists with different selectivity profile, induce anti-allodynic effects in one of the most relevant neuropathic pain model. In addition the expression of AgRP in spinal cord and DRG suggests an endogenous tonic inhibitory control on MC system activity. In pathological conditions this steady control could be insufficient to cope with an over activated MC system leading to increase in nociception. These data suggest that targeting MC4 with synthetic antagonists could restore the balance and hence reduce nociception.

http://www.sciencedirect.com/science/article/B6SYP-4CYP0Y4-2/2/7cd5ccbf73e4cf972e9d1cb588713f01

Previous studies in humans have demonstrated a high co-morbidity between alcoholism and smoking. This co-morbidity between alcohol and nicotine dependence can be attributed, in part, to common genetic factors. In rodents, behavioral and physiological responses to alcohol and nicotine also appear to share common genetic influences. In this report, the genetic correlation between free-choice oral nicotine and oral alcohol consumption was evaluated using an ascending two-bottle choice paradigm in C57BL/6 X C3H/HeJ F2 intercross mice. For all concentrations of nicotine (25, 50, and 100 [μg/ml] and alcohol (3, 6, and 10%) tested, nicotine consumption was significantly correlated with alcohol consumption. Nicotine consumption at the highest nicotine concentration tested (100 [μg/ml]) showed low, but significant, correlations with the number of [3H]-cytisine binding sites in the hippocampus ($r = 0.307$) and the number of [125I]-[alpha]-bungarotoxin binding sites in the cortex ($r = -0.328$). No significant correlations between alcohol consumption and the number of either [3H]-cytisine or [125I]-[alpha]-bungarotoxin binding sites was observed. A polymorphism in the nicotinic receptor [alpha]4 subunit gene, Chrna4, showed a trend with nicotine consumption and a significant association with alcohol consumption in female but not male mice. These results indicate that common genetic factors influence nicotine and alcohol consumption in mice. However, neither individual differences in the expression of [3H]-cytisine or [125I]-[alpha]-bungarotoxin binding nicotinic receptors nor the polymorphism in Chrna4 likely contribute to the genetic overlap that influences the consumption of both of these drugs of abuse in C57BL/6 X C3H/HeJ F2 mice.


http://www.sciencedirect.com/science/article/B6SYP-3W0P67V-2W/2/e0ef363c5beb8af756072d42356d9ae7

The differential sensitivity following the administration of [Delta]9-THC to 3 mouse strains, C57BL/6, DBA/2 and ICR mice, indicated that some of the neurobehavioral changes may be attributable to genetic differences. The objective of this study was to determine the extent to which the cannabinoid (CB1) receptor is involved in the observed behavioral changes following [Delta]9-THC administration. This objective was addressed by experiments using: (1) DNA-PCR and reverse PCR; (2) systemic administration of [Delta]9-THC, and; (3) intracerebral microinjection of [Delta]9-THC. The site specificity of action of [Delta]9-THC in the brain was determined using stereotoxic surgical approaches. The intracerebral microinjection of [Delta]9-THC into the nucleus accumbens was found to induce catalepsy, while injection of [Delta]9-THC into the central nucleus of amygdala resulted in the production of an anxiogenic-like response. Although the DNA-PCR data indicated that the CB1 gene appeared to be identical and intronless in all 3 mouse strains, the reverse PCR data showed two additional distinct CB1 mRNAs in the C57BL/6 mouse which also differed in pain sensitivity and rectal temperature changes following the administration of [Delta]9-THC. It is suggested that the diverse neurobehavioral alterations induced by [Delta]9-THC may not be mediated solely by the CB1 receptors in the brain and that the CB1 genes may not be uniform in the mouse strains.

http://www.sciencedirect.com/science/article/B6T4N-45BMCDFTY/2/5571ec7bbd93c6321ad212fb7ceea8


http://www.sciencedirect.com/science/article/B6WBN-4C4NSCN-3C/2/af9123b76c5bb8f87eece0eefd003e1a4

We analyzed DNA from 13 males with ornithine transcarbamylase (OTC) deficiency for gene deletions and known point mutations using the polymerase chain reaction (PCR), allele-specific oligonucleotide (ASO) hybridization, and Southern blotting with full-length OTC cDNA and exon-specific probes. Three patients were found to have deletions: one was missing the whole OTC gene; a second patient had a deletion of both exon 7 and 8; and the third had a deletion of exon 9. Only one of the remaining 10 patients had a known point mutation consisting of a G-to-A change in nucleotide 422 of the sense strand resulting in a glutamine substitution for arginine at amino acid 109 of the mature OTC protein. This study describes the integration of various molecular methods to screen OTC-deficient patients for deletions and point mutations. Two new deletions within the OTC gene are described.


http://www.sciencedirect.com/science/article/B6T4P-3YJYDBS-G/2/b151c4f5fb7ac58ca9ac9803d3ec3f28

The muscles of the corpus cavernosum of the penis relax in response to stimulation of non-adrenergic, non-cholinergic nerves or nitric oxide (NO)-donating drugs to elicit erection. It is generally assumed that NO mediates this effect via activation of soluble guanylyl cyclase and a
subsequent increase in cyclic guanosine 3',5'-monophosphate concentration. However, there are no data on the expression of this enzyme in human corpus cavernosum. The purpose of the present study was the molecular characterization of NO-sensitive guanylyl cyclase in human corpus cavernosum. RNA was extracted from tissue samples obtained from seven patients undergoing penile prosthetic surgery or correction of penile deviation. Reverse transcriptase-polymerase chain reaction (RT-PCR) with specific primers for the subunits of NO-sensitive guanylyl cyclase was performed, and PCR products were subcloned and sequenced. Specific amplification products encoding the [alpha]1, [beta]1, [alpha]2, and [beta]2 subunits were detected. In addition, we isolated a transcript encoding a novel variant [beta]2 subunit. To test whether this novel transcript arises by alternative splicing or whether it is encoded by a separate gene, a 4000-bp clone of the corresponding genomic DNA sequence was isolated. Sequence analysis suggests that the novel [beta]2 variant arises by alternative splicing from the same gene as the [beta]2 subunit on chromosome 13. In conclusion, our findings suggest the presence of different subunit mRNAs of NO-sensitive guanylyl cyclase in human corpus cavernosum.


http://www.sciencedirect.com/science/article/B6T4P-4754BR7-X8/2/233a213b2f8e3e4eb07a99ebeb3c2bf28

Stably expressed human and rat phenol UDP-glucuronosyltransferases (UGTs) of the UGT1 complex (HlugP1, HlugP4 and 3-methylcholanthrene-inducible rat UGT1A1, the latter considered to be an orthologous enzyme to HlugP1) have been used to investigate the role of UGTs in paracetamol glucuronidation. Kinetic analysis of recombinant UGTs was compared to that of total UGT activities in liver microsomes. Paracetamol was found to be an overlapping substrate of several UGTs. It shows higher affinity for HlugP1 and rat UGT1A1 (apparent Km values of 2 and 3 mM, respectively) than for HlugP4 (Km = 50mM) and other UGTs present in liver microsomes (Km values of > 12mM). Glucuronidation of paracetamol with HlugP1 contrasts with that of 6-hydroxychrysene and of 4-methylumbelliferone, which are conjugated with higher affinity by HlugP4 than by HlugP1. Due to the wide tissue distribution of rat UGT1A1, paracetamol glucuronidation was also investigated in extra-hepatic rat and human tissues. Paracetamol UGT activity was present and inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat kidney, lung and spleen. It was also detected in human kidney. A selective cDNA probe for exon 1 of HlugP1 cross-reacted with mRNA from both human liver and kidney. The results demonstrate that paracetamol is conjugated by HlugP1 and its rat orthologue UGT1A1 with higher affinity than by HlugP4 and other UGTs.


http://www.sciencedirect.com/science/article/B6T4P-3YYTD3V-G/2/57687f58ed8b785937fa2f8a113cd0fbb7

UDP-glucuronosyltransferases (UGTs), in addition to their role in overall pharmacokinetics, play important roles in local protection of cells against toxins and in the control of endogenous receptor ligands. UGT1A6, which conjugates planar phenols, appears to be expressed in many organs, but information on cell-specific expression in these organs is controversial or absent. Therefore, a non-isotopic in situ hybridization method was developed and applied to localize UGT1A6 expression in rat testis and brain. It was found that UGT1A6 is expressed in Sertoli cells and spermatogonia of rat testis and in brain neurons, in particular in hippocampal pyramidal cells and Purkinje cells of the cerebellum.

http://www.sciencedirect.com/science/article/B6T4P-3XRY8R1-9/2/70c5c6e938975184a4a34f1a165df36d

Arylamine N-acetyltransferase 1 (NAT1) conjugates several aromatic amines and their N-hydroxylated metabolites by N- or O-acetylation. NAT1 genotype and phenotype is known to be variable in human populations. In this study, we set out to measure the functional relevance of the frequent NAT1 gene variants for the activity in human red blood cells. Healthy German volunteers (N = 314) were genotyped for NAT1 alleles *3, *4, *10, *11, *14, and *15 using polymerase chain reactions and restriction fragment length pattern analysis, and NAT1 enzyme kinetic parameters were measured in a subset of 105 individuals using p-aminobenzoic acid as specific substrate. There was no functional difference between NAT1 alleles *4 and *10. In particular, there was no trend of increasing activity from NAT1*4/*4 to *4/*10 and *10/*10. Carriers of the NAT1*11 and *14 alleles had a statistically significant lower enzyme activity compared with carriers of the *3, *4, or *10 alleles. Compared with the wild-type genotype NAT1*4/*4, activity of the NAT1*11/*11, NAT1*11/*10, and NAT1*11/*4 genotypes was reduced by 20.7%, 35.7%, and 31.5%, respectively. Activity of the NAT1*10/*14 and NAT1*4/*14 genotypes was reduced by 49.8% and 55.6%, respectively. The difference in NAT1 activity between the *4/*11 and *4/*14 genotypes was also significant (P NAT1*15/*15 genotype had no detectable enzyme activity. In conclusion, functional consequences of NAT1 mutations were tested in a large population. Activity in carriers of NAT1 alleles *3, *4, and *10 did not differ, alleles NAT1*11 and *14 appeared to be low activity alleles, and allele NAT1*15 had no activity.


http://www.sciencedirect.com/science/article/B6T4P-4BRCNCP-B/2/29d708b3a8ba9f45ff7aad84e1b33917

Screening of a foetal brain genomic DNA library allowed to isolate a 10-kb fragment of the gene encoding the human [alpha]2B-adrenergic receptor, that contained 5.5 kb of the 5'-flanking region, the open reading frame and 2.9 kb of the 3'-flanking region. The 1-kb fragment upstream from the start codon was rich in GC, lacked consensus TATA or CAAT box, but contained several Sp1-binding sites. Other potential cis-regulatory elements found in the 5'-flanking region included AP2, USF, Stat-6, NF[alpha]B and Olf-1. A single canonical polyadenylation signal (AATAAA) was found at position +3252/+3257 and the polyadenylation site was 3274 nucleotides downstream from ATG. Transfection experiments with chimeric luciferase contructs containing various truncated fragments of the 5'-region showed that the fragment -3160/+3 exhibited promoter activity in all tested cell lines and permitted the definition of a minimal 200-bp promoter (-603/-411) containing three putative Sp1-binding sites and two initiator elements. Transcriptional activity of this region was inhibited by the addition of mithramycin, a specific inhibitor of Sp1 binding to GC-rich sequences. The search for sequence variants within a fragment covering 1.7 kb of 5'-flanking region and the coding region allowed us to identify five novel single nucleotide polymorphisms. Interestingly, the G/C substitution at position -98 relative to the start codon was common and in complete linkage with a previously identified insertion/deletion polymorphism in the coding region which was showed to affect [alpha]2B-adrenergic receptor function. Based on transfection data and computer-assisted sequence analysis, the -98 G/C single nucleotide polymorphism was located within a portion of the 5'-UTR (-127/+3) affecting luciferase activity and it created additional putative binding site for Sp1. However, G/C substitution had no
significant incidence on promoter activity in BHK-21 or HeLa cells.


The induction of cytochrome P450 3A (CYP3A) protein and mRNA by RU486 [17(beta)-hydroxy-11[beta]-(4-dimethylaminophenyl)-17[alpha]-1-propyl-estra-4,9-dien-3-one] treatment and food deprivation in female rat liver was studied using Western blotting and competitive reverse transcription-polymerase chain reaction (RT-PCR). CYP3A apoprotein levels increased in response to food deprivation and to RU486 treatment, and the combination of RU486 treatment plus food deprivation had an apparent additive effect. Food deprivation and RU486 treatment also caused increases in CYP3A1, CYP3A18, and CYP3A23 mRNA, and the combined effects of these treatments on each of these mRNA forms were synergistic. CYP3A2 mRNA was not detected in any of the treatment groups, and there was a lack of concordance between CYP3A9 mRNA levels and the specific messages corresponding to the other CYP3A isoforms. CYP3A9 mRNA levels were highest in food-deprived animals, whereas RU486 inhibited CYP3A9 mRNA expression and suppressed the induction effect of food deprivation. Food deprivation and RU486 treatment each separately caused increased microsomal diazepam C3-hydroxylase activity, and the combined effects of these treatments on this monooxygenase were additive. In contrast, the [N-methyl-14C]erythromycin demethylase activity of the fasted, RU486-treated group of rats did not differ from that of the untreated group, and kinetic analyses revealed that both groups of animals exhibited similar Km and Vmax values. These results suggest that CYP3A9 may be primarily responsible for erythromycin N-demethylation and that the isoforms induced by the combination of fasting and RU486 administration are CYP3A1, CYP3A23, and, to a lesser extent, CYP3A18.


The activity, expression and localization of the UDP-glucuronosyltransferases (UGTs) were investigated in human placenta at term. UGT activity (measured with the substrate 4-methylumbelliferone (4-MU)) was observed in all 25 placentas sampled and maximum velocity (Vmax) ranged 13-fold from 5.1+/-.0 to 66.9+/-.17.5 nmol/min/mg protein (mean+/-.SD). Substrate affinity (Km) ranged 5-fold from 246+/-.24 to 1124+/-.422 [mu]M. Using reverse transcriptase-polymerase chain reaction (RT-PCR), expression of the isoforms UGT2B4, 2B7, 2B10, 2B11 and 2B15 was observed in all (12/12) placentas sampled and expression of UGT2B17 was noted in 8/12 placentas. Northern analysis of the UGT2B7 isoform in 12 placentas revealed a 10-fold difference in expression with RT-PCR variability and the 13-fold variation observed in UGT activity. The presence of UGT2B4 and 2B7 proteins (52 and 56 kDa, respectively) was demonstrated by Western blotting. The sites of placental UGT2B transcription (in situ hybridization) and protein expression (immunohistochemistry) were located in the syncytium of the placental trophoblasts bordering the placental villi. UGT1A proteins could not be observed with immunohistochemistry or Western blotting and expression could not be observed with RT-PCR. Our discovery of UGT expression and activity at the site of maternal-fetal exchange is consistent with a role for UGTs in detoxification of exogenous and endogenous ligands and the
maintenance of placental function through clearance and regulation of steroid hormones.

Dabholkar, M., K. Thornton, et al. (2000). "Increased mRNA levels of xeroderma pigmentosum complementation group B (XPB) and Cockayne's syndrome complementation group B (CSB) without increased mRNA levels of multidrug-resistance gene (MDR1) or metallothionein-II (MT-II) in platinum-resistant human ovarian cancer tissues." Biochemical Pharmacology 60(11): 1611.


Tumor tissue specimens from human ovarian cancer patients were assessed for relative mRNA abundance levels of several genes thought to be involved in the development of in vitro drug resistance in this disease. Higher mRNA levels of Xeroderma pigmentosum group B (XPB), which links DNA repair with DNA transcription, and of Cockayne's syndrome group B (CSB), which is essential for gene-specific repair, were observed in tumor tissues that were clinically resistant to platinum-based chemotherapy, as compared with tissues from patients responding to therapy. In a cohort of 27 patients, mRNA levels of XPB averaged 5-fold higher in platinum-resistant tumors (P = 0.001); and for CSB, mRNA levels averaged 6-fold higher but with greater variability (P = 0.033). Concurrently, these platinum-resistant tumor tissues did not exhibit significantly higher mRNA levels for the MDR1 (multidrug-resistance) gene (P = 0.134) or of the metallothionein-II (MT-II) gene (P = 0.598). Since these platinum-resistant tumors also show higher mRNA levels of ERCC1 and XPA, platinum resistance appears to be associated with concurrent up-regulation of four genes (XPA, ERCC1, XPB, and CSB). These four genes participate in DNA damage excision activity, gene-specific repair, and linkage of DNA repair with DNA transcription. These data suggest that concurrent up-regulation of genes involved in nucleotide excision repair may be important in clinical resistance to platinum-based chemotherapy in this disease.


http://www.sciencedirect.com/science/article/B6T4P-48JK73P-1/2/9163e3a2a0a8c213ea96269be37451c9

We carried out a time-course study on the effects of a single intramuscular (i.m.) dose (0.5 x 50) of sarin (O-isopropyl methylphosphonofluoridate), also known as nerve agent GB, on the mRNA expression of acetylcholinesterase (AChE) in the brain of male Sprague-Dawley rats. Sarin inactivates the enzyme AChE which is responsible for the breakdown of the neurotransmitter acetylcholine (ACh), leading to its accumulation at ACh receptors and overstimulation of the cholinergic system. Rats were treated with 50 [mu]g/kg of sarin (0.5 x 50) in 1 mL saline/kg and terminated at the following time points: 1 and 2 hr and 1, 3, and 7 days post-treatment. Control rats were treated with normal saline. Total RNA was extracted, and northern blots were hybridized with cDNA probes for AChE and 28S RNA (control). Poly-A RNA from both treated and control cortex was used for reverse transcription-polymerase chain reaction (RT-PCR)-based verification of the data from the northern blots. The results obtained indicate that a single (i.m.) dose of sarin (0.5 x 50) produced differential induction and persistence of AChE mRNA levels in different regions of the brain. Immediate induction of AChE transcripts was noted in the brainstem (126+/-6%), cortex (149+/-4%), midbrain (153+/-5%), and cerebellum (234+/-2%) at 1 hr. The AChE expression level, however, increased over time and remained elevated after a decline at 1 day in the previously shown more susceptible brainstem. The transcript levels remained elevated at a later time point (3 days) in the midbrain, after a dramatic decline at day 1 (110+/-2%). In the cortex, transcript levels came down to control values by day 1. The cerebellum also showed a
decline of the elevated levels observed at 2 hr (275+/−2%) to control values by day 1. RT-PCR analysis of the AChE transcript at 30 min in the cortex showed an induction to 213+/−3% of the control level, confirming the expression pattern obtained by the northern blot data. The immediate induction followed by the complex pattern of the AChE mRNA time-course in the CNS may indicate that the activation of both cholinergic-related and unrelated functions of the gene plays an important role in the pathological manifestations of sarin-induced neurotoxicity.


http://www.sciencedirect.com/science/article/B6T4P-3W788WD-9/2/9c91d9f6ec37096eac51809f9816fb66

The effect of P-glycoprotein inhibition on the uptake of the HIV type 1 protease inhibitor saquinavir into brain capillary endothelial cells was studied using porcine primary brain capillary endothelial cell monolayers as an in vitro test system. As confirmed by polymerase chain reaction and Western blot analysis, this system functionally expressed class I P-glycoprotein (pgp1A). P-Glycoprotein isoforms pgp1B or pgp1D could not be detected. The uptake of saquinavir into endothelial cells could be described as the result of a diffusional term of uptake and an oppositely directed saturable extrusion process. Net uptake of saquinavir into cultured brain endothelial cells could be increased significantly up to 2-fold by SDZ PSC 833 in a dose-dependent manner, with an 50 of 1.13 [μM]. In addition, the HIV protease inhibitor ritonavir inhibited p-glycoprotein-mediated extrusion of saquinavir with an 50 of 0.2 [μM], indicating a high affinity of ritonavir for p-glycoprotein. In conclusion, we showed that the HIV protease inhibitor ritonavir is a more potent inhibitor of P-glycoprotein than the multidrug resistance (MDR)-reversing agent SDZ PSC 833. The inclusion of this drug in combination regimens may greatly facilitate brain uptake of HIV protease inhibitors, which is especially important in patients suffering from AIDS dementia complex.


http://www.sciencedirect.com/science/article/B6T4P-3X6SBXC-9/2/755c9f49e9de93bbf0c5833d9bada326

Nitric oxide (NO) produced in endothelial cells has been implicated in the regulation of blood pressure, regional blood flow, inhibition of platelet aggregation, and endothelial and vascular smooth muscle cell proliferation. In a variety of cardiovascular disease states, such as atherosclerosis, arterial hypertension, and restenosis, expression of endothelial NO synthase (NOS-III) and endothelial NO production appear to be altered. Thus, NOS-III is an attractive target for cardiovascular gene therapy for which adenoviral vectors are one of the most effective vector systems. Therefore, a recombinant adenoviral vector expressing NOS-III (adenovirus type 5 [Ad5] cytomegalovirus [CMV] NOSIII) was constructed and biochemically and pharmacologically characterized both in vitro and in intact cells. Ad5CMVNOSIII-derived recombinant NOS-III was successfully expressed, as shown by immunoprecipitation and immunocytochemistry, and biologically active, as shown by functional assays in human primary umbilical vein and EA.hy926 endothelial cells, as well as 293 human embryonic kidney and Chinese hamster ovary cells. The Km values for NADPH and -arginine and the Ka for tetrahydrobiopterin as well as the enzyme’s dependency on other cofactors were similar to recombinant reference enzyme and literature values. NOS-III expression levels correlated linearly
with the multiplicity of infection with Ad5CMVNOSIII and lasted for at least 8 days. NOS-III transfection inhibited endothelial cell proliferation. In conclusion, adenovirus-mediated gene transfer of Ad5CMVNOSIII to vascular and non-vascular cells resulted in the dose-dependent expression of intact, physiologically regulated, and functionally active NOS-III.


http://www.sciencedirect.com/science/article/B6T4P-462BRBX-9/2/a394466934fa28d45b3a1fbd6ea56d52

Continuous cultivation of T-lymphoid H9 cells in the presence of 3′-azido-2′,3′-dideoxythymidine (AZT) resulted in a cell variant cross-resistant to both thymidine and deoxycytidine analogs. Cytotoxic effects of AZT, 2′,3′-didehydro-3′-deoxythymidine as well as different deoxycytidine analogs such as 2′,3′-dideoxyctydine, 2′,2′-difluoro-2′-deoxyctydine (dFdC) and 1-[ss]-D-arabinofuranosylcytosine (ara-C) were strongly reduced in H9 cells continuously exposed to AZT when compared to parental cells (>8.3-, >6.6-, >9.1-, 5 x 104-, 5 x 103-fold, respectively). Moreover, anti-HIV-1 effects of AZT, d4T, ddC and 2′,3′-dideoxy-3′-thiacytidine (3TC) were significantly diminished (>222-, >25-, >400-, >200-fold, respectively) in AZT-resistant H9 cells. Study of cellular mechanisms responsible for cross-resistance to pyrimidine analogs in AZT-resistant H9 cells revealed decreased mRNA levels of thymidine kinase 1 (TK1) and lack of deoxycytidine kinase (dCK) mRNA expression. The loss of dCK gene expression was confirmed by western blot analysis of dCK protein as well as dCK enzyme activity assay. Moreover, enzyme activity of TK1 and TK2 was reduced in AZT-resistant cells. In order to determine whether lack of dCK affected the formation of the active triphosphate of the deoxycytidine analog dFdC, dFdCTP accumulation and retention was measured in H9 parental and AZT-resistant cells after exposure to 1 and 10 [μM dFdC. Parental H9 cells accumulated about 30 and 100 pmol dFdCTP/106 cells after 4 hr, whereas in AZT-resistant cells no dFdCTP accumulation was detected. These results demonstrate that continuous treatment of H9 cells in the presence of AZT selected for a thymidine analog resistant cell variant with cross-resistance to deoxycytidine analogs, due to deficiency in TK1, TK2, and dCK.


http://www.sciencedirect.com/science/article/B6T4P-47724F9-1Y2/7890cb76dd712de6b243b7a753798f1

The p80cdc25 protein is a protein phosphatase directly involved in p34cdc2 protein kinase activation by dephosphorylation. The cdc25B gene is one of three human cdc25 homologs which can complement the temperature-sensitive cdc25 mutation of Schizosaccharomyces pombe, and is expressed at high levels in human cell lines, particularly in some cancer cells. A fusion protein of glutathione-S-transferase (GST) and the catalytic domain of cdc25B protein was constructed and found to retain phosphatase activity in the manner of a p80cdc25 phosphatase by using a chromogenic substrate, p-nitrophenylphosphate. Two benzoquinoid antitumor compounds, dnacin A1 and dnacin B1, inhibited phosphatase activity in a non-competitive manner.

Horiguchi, T. and S. Tanida (1995). "Rescue of Schizosaccharomyces pombe from camptothecin-
mediated death by a DNA topoisomerase I inhibitor, TAN-1518 A. " Biochemical Pharmacology 49(10): 1395.

http://www.sciencedirect.com/science/article/B6T4P-3YVDRK0-1J/2/f72e3ed71bdc942c629afe7259839804

TAN-1518 A is a cytotoxic agent with suppressive activity against Meth A fibrosarcoma in vivo. This compound inhibits calf thymus DNA topoisomerase I (Topo I) but does not stimulate cleavable complex formation in the nuclei of Chinese hamster ovary (CHO)-K1 cells, suggesting that it inhibits Topo I in a manner different from that of camptothecin (CPT). To clarify the mode of action of TAN-1518 A, we examined its effects on the eukaryotic microorganism Schizosaccharomyces pombe (S. pombe), which does not require Topo I as an essential factor for growth. TAN-1518 A inhibited purified S. pombe Topo I as potently as did CPT. TAN-1518 A, unlike CPT, did not stimulate Topo I-induced DNA cleavage; instead, it inhibited CPT-induced cleavable complex formation. We constructed a S. pombe strain, IR9, that produced excess Topo I. IR9 was hypersensitive to CPT, but its growth was not affected by TAN-1518 A. The CPT-mediated death of IR9 cells was reduced dramatically in the presence of TAN-1518 A. These findings clearly demonstrate that TAN-1518 A is a specific inhibitor of Topo I in eukaryotic cells and also suggest that this agent inhibits some earlier step(s) that occurs before the formation of cleavable complex on DNA strands in the catalytic cycle of this enzyme.


http://www.sciencedirect.com/science/article/B6T4P-43T1MD9-C/2/5d7b126b6d0d277b9221cdf345ff26de

The levels and activities of pulmonary microsomal CYP1A1 and CYP1A2 in 40-day-old male and female, and 120-day-old male offspring of pregnant rats treated with five weekly 0.1 [μg/kg doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during gestation and lactation were compared with those in age-matched offspring of untreated dams. The CYP1A1-preferential activity, ethoxyresorufin O-deethylase (EROD), was comparably induced 5.3- and 6.4-fold in 40-day-old male and female offspring, respectively, but was not induced in 120-day-old male offspring, of TCDD-treated dams. Similarly, CYP1A1 protein was induced in 40-day-old female or male offspring of untreated dams but was undetectable in 120-day-old offspring of untreated or treated dams. CYP1A2 activity, as measured by the bioactivation of 2-amino-3,4-dimethylimidazol[4,5-f]quinoline (MeIQ) to mutagens in the Ames assay, was elevated 11.1- and 5.5-fold in 40-day-old female and male offspring, respectively, of TCDD-treated dams, but was unaffected by TCDD exposure in 120-day-old offspring. CYP1A2 protein was undetectable in 40-day-old male or female offspring of untreated dams or in 120-day-old male offspring of treated or untreated dams; it was detected in 40-day-old offspring of treated dams, at a level that was higher in females than in males. The results show that gestational and lactational exposure to TCDD causes long-lasting and gender-preferential induction of CYP1A1 as well as CYP1A2 in the lungs of rat offspring.


http://www.sciencedirect.com/science/article/B6T4P-3YW28BX-C/2/e7365c183910b9ebc865dce7041bb8df
The present study investigated the role of growth hormone (GH) in hepatic CYP3A18 and CYP3A9 expression in prepubertal and adult male rats. For comparison, the effects of GH on CYP3A2 expression were also measured. Initial experiments demonstrated that CYP3A18 mRNA levels were greater during puberty and adulthood than during the prepubertal period, CYP3A9 mRNA was not expressed until puberty and its expression increased in adulthood, and CYP3A2 mRNA levels were relatively constant from prepuberty to adult life. Hypophysectomy, which results in the loss of multiple pituitary factors including GH, increased CYP3A2 and CYP3A18 mRNA expression 3- to 4-fold, but it did not affect CYP3A9 mRNA levels or CYP3A-mediated testosterone 2[beta]- or 6[beta]-hydroxylase activity in adult rats. GH administered as twice daily s.c. injections (0.12 [mu]g/g body weight) to hypophysectomized or intact adult rats did not affect CYP3A18 or CYP3A9 mRNA expression. The same treatment decreased CYP3A2 mRNA and protein and testosterone 2[beta]- and 6[beta]-hydroxylase activity levels in intact but not hypophysectomized rats. However, in intact prepubertal rats, intermittent GH administration decreased CYP3A18 and CYP3A2 mRNA levels, but a higher dosage (3.6 [mu]g/g) was required to suppress CYP3A2. Overall, the present study demonstrated that: (a) the constitutive expression of CYP3A18, CYP3A9, and CYP3A2 does not require the presence of GH, (b) CYP3A18 is more sensitive than CYP3A9 to GH modulation in adult rats; and (c) CYP3A2 is less sensitive to the suppressive influence of GH during the prepubertal period than during adult life.


http://www.sciencedirect.com/science/article/B6T4P-47795G1-R7/2/3825282594ece34cff8b045f937b8ed6

Cyclooxygenase (COX), a key enzyme in the formation of prostanoids, is known to exist in two isoforms: an inducible enzyme (COX 2) and a constitutive form (COX 1). Both enzymes are inhibited by non-steroidal anti-inflammatory drugs (NSAID), but only marginal selectivity has thus far been reported. In this study, we report on a novel selective inhibitor of COX 2, CGP 28238 (6-(2,4-dinuorophenoxy)-5-methyl-sulfonylamino-1-indanone). Human washed platelets were used as a source of COX 1. For IL-1 stimulated rat mesangial cells we demonstrated the almost exclusive presence of COX 2 in western blot and mRNA analysis. Therefore these two model systems were chosen for selectivity testing. With an 50 value of 15 nM, CGP 28238 blocked COX 2 activity in a similar concentration range to that of other potent NSAID such as indomethacin and diclofenac (50 = 1.17-8.9 nM). However, in contrast to these reference NSAIDs, CGP 28238 was at least 1000-fold less potent in inhibiting COX 1. Using other cell systems reported to express COX 1 or COX 2, we obtained a similar selectivity for COX 2. Thus, on the basis of our findings, CGP 28238 is a novel, highly potent and selective inhibitor of COX 2 and may be a lead compound for a new generation of potent anti-inflammatory drugs with an improved side-effect profile.


Caco-2 cells are a widely used model in drug development to study intestinal drug transport and metabolism. Recently, serotonin (5-hydroxytryptamine, 5-HT) has been characterized as a highly selective substrate of human UDP-glucuronosyltransferase UGT1A6 [Krishnaswamy S, Duan SX, von Moltke LL, Greenblatt DJ, Court MH. Validation of serotonin (5-hydroxytryptamine) as an in vitro substrate probe for human UDP-glucuronosyltransferase (UGT) 1A6. Drug Metab Disp 2003; 31:133-9], an isoform which conjugates planar phenols and is inducible by Ah receptor agonists
and by oxidative/electrophile stress. To gain more insight into intestinal 5-HT disposition, uptake and metabolism of this neurotransmitter was studied in Caco-2 cell monolayers. It was found that 5-HT was taken up from the basolateral and to a lesser extent from the apical surface. It was mainly excreted basolaterally as 5-HT glucuronide. 5-HT UGT activity and UGT1A6 mRNA were induced by Ah receptor agonists and by oxidative stress generated by tert-butylhydroquinone and by isomeric thymoquinone, a potential antitumor agent and constituent of Nigella sativa seeds, commonly used as a condiment in the Middle East. While UGT1A6 induction was clearly detectable in NAD(P)H:quinone oxidoreductase 1 (NQO1)-deficient Caco-2 cells, it was not induced in NQO1-efficient HT-29 colon adenocarcinoma cells. The results suggest that - in addition to its detoxification function - intestinal UGT1A6 contributes to intestinal homeostasis of 5-HT from dietary sources and from release by enterochromaffin cells.

Kohle, C., B. Mohrle, et al. (2003). "Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians." Biochemical Pharmacology 65(9): 1521.

http://www.sciencedirect.com/science/article/B6T4P-488GB0P-1/2/d2ef1c022f0a6b4bfffe9f98202b16

Polymorphisms of drug metabolizing enzymes are frequently associated with diseases and side effects of drugs. Recently, a TATA box mutation of UGT1A1 (UGT1A1*28), a common genotype leading to Gilbert's syndrome, and several missense mutations of other UDP-glucuronosyltransferase 1 (UGT1) family members have been described. Furthermore, co-occurrence of UGT1A1*28 and UGT1A6*2 has been observed. In order to elucidate the basis for co-occurrence of UGT1 mutations, fluorescence resonance energy transfer techniques were developed for rapid determination of polymorphisms of three UGT isoforms (UGT1A1*28, 1A6*2, and 1A7*2/*3). Hundred healthy Caucasians and 50 Egyptians were genotyped. All genotypes followed the Hardy-Weinberg equilibrium. Only three major haplotypes were found, including a haplotype consisting of allelic variants of all three isoforms (29% in Caucasians and 22% in Egyptians), all leading to reduced UGT activity. Frequent haplotypes containing several UGT1 allelic variants should be taken into account in studies on the association between diseases, abnormal drug reactions, and UGT1 family polymorphisms.


http://www.sciencedirect.com/science/article/B6T4P-3TCW06S-G/2/db93dbb6a8756de8ef921e6b2a17a29e

The coincidence of mutated alleles of CYP2C18 and CYP2C19 was studied in 154 Japanese subjects. The mutant alleles of CYP2C18 studied were CYP2C18m1 (T204 -> A substitution in exon 2) and CYP2C18mFR (A-460 -> T substitution in the 5'-flanking region), and those of CYP2C19 were CYP2C19m1 (G689 -> A substitution in exon 5) and CYP2C19m2 (G636 -> A substitution in exon 4). They were identified by polymerase chain reaction and restriction fragment length polymorphism. The results indicate that genotypes of CYP2C18m1 and CYP2C18mFR are completely coincident with those of CYP2C19m2 and CYP2C19m1, respectively. The finding suggests that the mutations of CYP2C18 and CYP2C19 examined in the present study are very closely linked with each other (i.e. CYP2C18m1 vs CYP2C19m2 and CYP2C18mFR vs CYP2C19m1), at least in a Japanese population.

http://www.sciencedirect.com/science/article/B6T4P-3VW752G-G/2/22b3868497f26d25f3efac5b75532e6c

The cytochromes P450 are a large family of haemoproteins which have a major role in the oxidative metabolism of a wide range of xenobiotics and some endogenous compounds. In this study the presence of individual members of the CYP1, CYP2 and CYP3 P450 families has been investigated by reverse transcriptase polymerase chain reaction in different regions of normal human brain consisting of frontal and temporal cortices, mid brain, cerebellum, pons and medulla. All the P450s were identified in specific regions of brain with CYP1A1 and CYP2C being the most frequently expressed forms of P450. Sequencing identified the CYP2C PCR product as CYP2C8. This study indicates that individual P450 mRNAs are present in human brain and are found in specific brain regions. The distribution of individual P450s in different regions of human brain is likely to be highly important in determining the response of the brain to toxic foreign compounds.


http://www.sciencedirect.com/science/article/B6T4P-48FCJWV-Z/2/bc0ec075063967b03ab8922aa6058ccb

Cytochrome P450 (CYP) drug metabolising enzymes CYP1A1 and CYP1B1 are regulated through the ligand-activated aryl hydrocarbon (Ah) receptor. Differential expression of CYP1A1 and CYP1B1 mRNA and protein has previously been reported in human tissues with the presence of the message often extrapolated to indicate the presence of protein. The aim of this study was to clarify these potentially misleading findings, by analysing components of the Ah receptor pathway (CYP1B1, CYP1A1, Ah receptor and ARNT) using a combination of quantitative real-time RT-PCR and immunoblotting. Three human cell lines (MOG-G-CCM, MCF7 and HEPG2) known to differentially express CYP1A1 and CYP1B1 mRNA and protein were exposed to the Ah receptor agonist 3-MC, and basal and inducible levels of CYP1A1, CYP1B1, Ah receptor and ARNT were determined. The key finding of this study was the demonstration of equivalent levels of CYP1B1 mRNA in both the treated and untreated MOG-G-CCM cell lines, with expression of the corresponding CYP1B1 protein only after exposure to an Ah receptor agonist. This finding suggests that a post-transcriptional mechanism is involved in the regulation of CYP1B1. In addition, the expression pattern of CYP1B1 mRNA and protein in the MOG-G-CCM cells highlights this cell line as a potential model for studying CYP1B1 expression in human tissue.


http://www.sciencedirect.com/science/article/B6T4P-42D81KP-C/2/2a382a799e4dc95fa73c21b0300163b1

Ribonuclease H (RNase H), an enzyme that cleaves an RNA sequence base-paired with a complementary DNA sequence, is proposed to be the mediator of antisense phosphorothioate
oligonucleotide (S-oligo) lethality in a cell. To understand the role of RNase H in the killing of the parasitic protozoan Leishmania by antisense S-oligos, we expressed an episomal copy of the Trypanosoma brucei RNase H1 gene inside L. amazonensis promastigotes and amastigotes that constitutively express firefly luciferase. Our hypothesis was that S-oligo-directed degradation of target mRNA is facilitated in a cell that has higher RNase H activity. Increased inhibition of luciferase mRNA expression by anti-luciferase S-oligo and by anti-miniexon S-oligo in these stably transfected promastigotes overexpressing RNase H1 was correlated to the higher activity of RNase H in these cells. The efficiency of killing of the RNase H overexpressing amastigotes inside L. amazonensis-infected macrophages by anti-miniexon S-oligo was higher than in the control cells. Thus, RNase H appears to play an important role in the antisense S-oligo-mediated killing of Leishmania. Chemical modification of S-oligos that stimulate RNase H and/or co-treatment of cells with an activator of RNase H may be useful for developing an antisense approach against leishmaniasis. The transgenic Leishmania cells overexpressing RNase H should be a good model system for the antisense-mediated gene expression ablation studies in these parasites.


http://www.sciencedirect.com/science/article/B6T4P-3RJPBNK-5/2/502410c1e0b7196243353e6330f84c7

We have examined the expression of three prostaglandin (PG) receptors, EP2, EP4, and FP, in a nonpigmented ciliary epithelial cell line (ODMC1-2) and in human ciliary muscle (HCM) cells. Total RNA preparations from either ODMC1-2 or HCM cells were subjected to reverse transcription-polymerase chain reaction (RT-PCR) with sense and antisense primers for each of the three PG receptors. The RT-PCR generated DNA products of predicted sizes corresponding to the EP2, EP4, and FP receptors in both ODMC1-2 and HCM cells. PCR products corresponding to each receptor were hybridized with specific 32P-labeled probes and, for further confirmation, digested with appropriate restriction enzymes. Pharmacological studies with the EP2 receptor-selective agonist butaprost resulted in a significant increase in the cyclic AMP level in ODMC1-2 cells. The stimulation of cyclic AMP in ODMC1-2 cells by PGE2 and 11-deoxy PGE1, the respective EP1/EP2/EP3/EP4 and EP2/EP3/EP4 receptor agonists, was concentration dependently inhibited by the EP4 receptor-selective antagonist AH23848. These results conclusively demonstrate the presence of both mRNA and protein for EP2, EP4, and FP receptors in ODMC1-2 and HCM cells.


http://www.sciencedirect.com/science/article/B6T4P-3VXH2BS-G/2/2e5e64a26371b9d9b2b69b3809f20202b9

The efficacy of taxol against a wide range of sensitive and refractory solid tumors has prompted extensive investigation into the factors that influence its cytotoxicity. Our preliminary observations indicated that taxol had a superior antitumor effect against human cells (Daudi, K562, 2008, 2008/C13*, 2780 and C70) compared with its effect against rodent cells (WS, WR, NIH3T3, and CHO). Although verapamil, an inhibitor of P-glycoprotein function, markedly increased the efficacy of taxol against the rodent cells (WS, WR, and CHO), the expression of P-glycoprotein was found only at low levels in the WR cells. In addition, levels of the multidrug resistance-associated protein (MRP), as assessed by reverse transcriptase-polymerase chain reaction
analysis, were found to be higher in the human than in the rodent cells, although MRP mRNA
was not detected by northern blotting. Transport studies indicated that the reduced sensitivity of
the rodent cells to taxol was due to decreased intracellular taxol levels and reduced intracellular
binding. However, no correlation was found between the intracellular binding of taxol and the
intracellular levels of [alpha]- and [beta]-tubulin, or the intracellular concentration of polymerized
tubulin. These studies were extended further by assessing the binding of taxol to semi-purified
microtubule proteins from WS, CHO and 2008/C13* cells in vitro. The microtubule protein
preparations from WS, CHO and 2008/C13* cells, which have a 50-fold difference in their
sensitivity to taxol, were found to bind equal amounts of radiolabeled taxol, and this binding was
inhibited (80%) in the presence of unlabeled taxol. These results lead us to propose the presence
in the rodent cells of an alternative taxol transport system that is distinct from the P-glycoprotein
and MRP systems.

Parekh, H., K. Wiesen, et al. (1997). "Acquisition of taxol resistance via P-glycoprotein- and non-P-
glycoprotein-mediated mechanisms in human ovarian carcinoma cells." Biochemical
http://www.sciencedirect.com/science/article/B6T4P-3RJ912M-3/2/a304bc8fede2992f4108e2945382bb9

Taxol-resistant clones from a human ovarian carcinoma cell line (2008) were selected by an initial
exposure to 0.05 [mu]M (2008/13) or 0.5 [mu]M (2008/17) taxol. Thereafter, a series of clones
with increasing taxol resistance were derived from the 2008/17 and 2008/13 cells by stepwise
sequential exposure to increasing concentrations of taxol. The 2008/17 clones displayed a
classical P-glycoprotein-mediated drug-resistance phenotype. In contrast, the 2008/13 clones
followed the classical P-glycoprotein-mediated resistance phenotype until a 245-fold taxol-
resistant clone (2008/13/2) was obtained, which was followed by a further increase in the degree
of resistance but significant down-regulation of P-glycoprotein expression in the 252-fold taxol-
resistant 2008/13/4 cells. This clone (2008/13/4) also accumulated significantly higher
intracellular levels of taxol than those expressing the P-glycoprotein. No correlation between the
expression of the multidrug resistance-associated protein and taxol resistance was observed.
Verapamil increased the sensitivity of all drug-resistant clones to taxol, and this was probably
related to the ability of verapamil to increase the intracellular concentration of taxol (except in the
case of 2008/13/4 cells). The 2008/17 clones were highly cross-resistant to Adriamycin(R),
etoposide, and vincristine. They also displayed a low level of cross-resistance to camptothecin
but were not cross-resistant to cisplatin. The taxol-resistant 2008/13 clones displayed a similar
pattern of crossresistance for all drugs (except Adriamycin). The 2008/13 clones were only 2- to
4-fold cross-resistant to Adriamycin. The levels of [alpha]-tubulin and [beta]-tubulin were similar in
the parental 2008 and taxol-resistant 2008/13/4 cells. Furthermore, the in vitro binding of
[3H]taxol to semipurified microtubule preparations derived from the parental 2008 and the taxol-
resistant 2008/13/2 and 2008/13/4 cells was similar. These results show that in human ovarian
carcinoma cells resistance to taxol can be acquired via as yet undescribed mechanisms.

low level of paclitaxel resistance in human ovarian and breast cancer cells." Biochemical
Pharmacology 63(6): 1149.
http://www.sciencedirect.com/science/article/B6T4P-45FYNH5-2/2/8250adc13e081033bf8db169a042757b

Paclitaxel, an antimitotic, anticancer agent, induces cell cycle arrest in the mitotic phase by
binding to the [beta]-tubulin subunit and forming highly stable microtubule polymers that resist
depolymerization. The overexpression of the P-glycoprotein (P-gp) and/or alteration in the cellular microtubules is associated with the development of paclitaxel resistance. However, we have established a paclitaxel-resistant human ovarian carcinoma subline (2008/13/4) wherein the degree of resistance could not be correlated with overexpression of P-gp, alterations in the [alpha]- and [beta]-tubulin isotypes, or changes in the drug-binding affinity of the microtubules. mRNA differential display analysis revealed the overexpression of sorcin, a calcium-binding protein in the 2008/13/4 cells. However, no detectable changes in the intracellular calcium levels were detected in the parental and the paclitaxel-resistant variant. Furthermore, co-treatment with A23187, a calcium ionophore, did not alter the cytotoxicity of paclitaxel against the parental and the paclitaxel-resistant cells. Transfection of the parental 2008 cells with full-length sorcin cDNA induced a low level (3-5-fold) of paclitaxel resistance. In addition, transfection of human breast cancer cells with the full-length sorcin cDNA also led to the induction of a low level of paclitaxel resistance in the transfecants. Although the overexpression of sorcin did not produce high levels of paclitaxel resistance, the results obtained present compelling evidence of the involvement of sorcin in developing low-level paclitaxel resistance in a variety of tumor cells. The precise biochemical mechanism(s) by which sorcin overexpression induces low-level paclitaxel resistance is currently under investigation.


http://www.sciencedirect.com/science/article/B6T4P-45Y04G7-1/2/8d56a21e4828d4aa53b6d1a41748d5a9

Exposure of cultured glomeruli to benzo[a]pyrene (BaP), a carcinogenic hydrocarbon, modulates mesangial and visceral epithelial cell proliferation in vivo and in vitro. The present studies were conducted to characterize mitogenic signaling profiles of cultured glomeruli following repeated cycles of BaP challenge. Enhanced rates of DNA synthesis were observed by the third passage in randomly cycling cultures after single or repeated carcinogen exposure. This response was characterized by upregulation of mitogenic sensitivity during early cell cycle transit, and increased cell numbers under restrictive growth conditions. The mitogenic response to platelet-derived growth factor (0.5 to 25 ng/mL), acidic fibroblast growth factor (2.5 to 10 ng/mL), basic fibroblast growth factor (0.05 to 5 ng/mL), epidermal growth factor (0.5 to 5 ng/mL), or conditioned medium was not enhanced by hydrocarbon challenge. BaP-treated cultures exhibited anchorage-independent growth and increased expression of hepatocyte growth factor mRNA and E-cadherin protein. Binding of activator protein-1 to DNA was enhanced in BaP-treated cells, but this change did not involve truncation or mutation of the c-jun delta region. Collectively, the data demonstrate that repeated cycles of BaP injury alter mitogenic signaling profiles in cultured glomerular cells. These alterations may contribute to deregulation of proliferative control following carcinogen exposure in vivo.


http://www.sciencedirect.com/science/article/B6T4P-3V394WK-6/2/4c5a7f0002ba5dd1209da0a9a4dc9c39

The discordance between P-glycoprotein (P-gp) expression and functionality [as measured by the efflux of doxorubicin (DOX)] was analyzed in a DOX-sensitive human breast cancer cell line (HTB-123) with high reactivity against four P-gp specific monoclonal antibodies (C219, MRK-16, UIC2, and 4E3). Reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting
analyses confirmed the overexpression of MDR1 mRNA and P-gp in this cell line. However, incubation of cells with efflux blockers, verapamil (VPL) or dipyridamole (DPD), did not enhance cellular (DOX) accumulation or cytotoxicity. Upon incubation with 12-O-tetradecanoylphorbol-13-acetate (TPA), HTB-123 cells retained less DOX than control cells and were sensitive to the efflux blockers verapamil or dipyridamole. These observations suggest that 12-O-tetradecanoylphorbol-13-acetate-induced P-gp phosphorylation may be associated with induction of P-gp-mediated drug efflux in the HTB-123 cell line.


http://www.sciencedirect.com/science/article/B6T4P-3YVDRS5-56/2/4b872a7f93820d37917101ea87ea28eb

The expression of drug resistance-associated mdr-1, GST [pi], and topoisomerase II genes was analyzed in cell cycle phase enriched populations of doxorubicin-resistant murine leukemic P388/R-84 cells. Flow cytometric analysis of bromodeoxyuridine (BrdU) incorporation and staining with anti-BrdU antibodies was used to confirm the purity of cell cycle phase enriched populations obtained by centrifugal elutriation. Doxorubicin (DOX) and daunorubicin (DNR) accumulation was significantly lower in S-phase cells, and coincubation with verapamil (VPL) or chlorpromazine (CPZ) enhanced DOX and DNR accumulation more in S-phase than in G1- and G2/M-phase cells. While the cellular content of mdr-1 and topoisomerase II mRNAs changed, GST [pi] mRNA content remained constant during the cell cycle. S-phase cells had about 3-fold higher mdr-1 mRNA content than G1- and G2/M-phase cells. In G1 cells, P-glycoprotein expression, as determined by C219 monoclonal antibody, was 12% less than that of S and G2/M cells. Topoisomerase II mRNA content increased with the progression of cell cycle and peaked in G2/M cells. These observations suggest that cell cycle stage related changes in expression of drug resistance markers may have a major bearing on chemosensitivity of drug-resistant cells.


http://www.sciencedirect.com/science/article/B6T4P-475TFC1-DJ/2/fde633c9a408a50e237c3ffbb26644051

The murine Cyp2a-4 and Cyp2a-5 genes encode P450 isoforms catalysing testosterone 15[alpha]-hydroxylase and coumarin 7-hydroxylase (COH) activities, respectively. Two days after the administration of a hepatotoxic dose of cerium chloride (2 mg/kg i.v.), COH activity was increased 3.2-fold in the liver of DBA/2 mice. Three and 4 days after the cerium treatment, coinciding with the occurrence of overt liver damage, there was a dramatic decrease in COH activity. The activities of testosterone 15[alpha]-hydroxylase and the Cypla-1-mediated 7-ethoxyresorufin O-deethylase (EROD) were decreased in response to cerium. Much less pronounced changes in the enzyme activities occurred in the C57BL/6 mouse liver. Northern blot analysis showed a 21-fold increase in the hepatic Cyp2a-4/5 mRNA in the DBA/2 mice at day 2, whereas no increase occurred in the C57BL/6 mice. Also in the kidneys the increase in COH activity and in Cyp2a-4/5 mRNA was marked only in the DBA/2 mice. A polymerase chain reaction-mediated analysis method utilizing a unique PstI restriction site in the Cyp2a-5 cDNA was used to differentiate between the highly homologous Cyp2a-4 and Cyp2a-5 mRNAs. Cerium was found to increase the amount of hepatic and renal Cyp2a-4 and Cyp2a-5 mRNA only in the DBA/2 mice. These data indicate that the Cyp2a-4/5 complex is regulated in a different way in DBA/2 and C57BL/6 mice and that some association exists between the development of liver
Troglitazone, a novel thiazolidinedione drug used to treat non-insulin-dependent diabetes mellitus, is a selective ligand for the peroxisome proliferator-activated receptor-[gamma] (PPAR[gamma]). Recent results indicate that PPAR[gamma] activation by thiazolidinediones regulates adipose tissue- and monocyte/peritoneal macrophage-derived cytokine expression in vitro. We evaluated whether troglitazone may also negatively regulate cytokine expression in the liver, which harbors the majority of the body's resident macrophages but which only weakly expresses PPAR[gamma]. Lean C57BL6 mice and genetically obese KKAy mice were chronically treated with troglitazone (100 mg/kg/day for 2 weeks). At the end of treatment, hepatic expression of tumor necrosis factor (TNF)-[alpha] and interleukin (IL)-6 mRNA was quantitatively determined by kinetic polymerase chain reaction both under basal conditions and after stimulation with lipopolysaccharide (LPS). Both untreated lean and obese mice exhibited low levels of baseline TNF-[alpha] and IL-6 mRNA expression and responded with a dramatic increase in hepatic cytokine transcripts and TNF-[alpha] protein expression following a challenge with LPS. Similar to the effects on white adipose tissue, troglitazone not only down-regulated the baseline levels of hepatic TNF-[alpha] and IL-6, but also greatly attenuated the inducing effects of LPS. The extent of this inhibitory effect of troglitazone was higher in obese KKAy mice than in lean mice and was also reflected by markedly down-regulated hepatic TNF-[alpha] mRNA expression. These data demonstrate that chronic administration of troglitazone is associated with a greatly attenuated responsiveness towards inducers of hepatic TNF-[alpha] and IL-6 production. The possible biological consequences of these effects, however, have not yet been assessed.

EO9, a new bioreductive indoloquinone alkylating agent, requires activation by a two-electron reduction, which can be catalysed by the NAD(P)H: quinone oxidoreductase DT-diaphorase (DTD) (EC 1.6.99.2). Seven human and four murine tumor cell lines from different histological origins were evaluated for their DTD enzyme activity (evaluated using dichlorophenolindophenol and EO9 as substrates), DTD gene expression and chemosensitivity to EO9. In general the cell lines could be divided into two groups: leukemic cells which were relatively resistant to EO9 (50 [ges] 0.5 [mu]M) and had no measurable DTD activity, and solid tumor cells, which were more sensitive to the drug (50 90 nmol/min/mg). The expression of the DTD gene was measured by semiquantitative PCR in the human cell lines and an excellent correlation between gene expression and enzyme activity was observed (r² = 0.94). A higher DTD gene expression also correlated with higher chemosensitivity to EO9. Protection of chemosensitivity to EO9 by dicoumarol, a strong and specific inhibitor of DTD activity, was dependent on duration of exposure and concentration of dicoumarol. Inhibition was best observed by short exposure to dicoumarol and EO9 together, demonstrating that bioactivation of EO9 by DTD is essential. In conclusion, DTD activity and expression appear to predict sensitivity to EO9 in a variety of cell lines.
lines. Evaluation of activity or expression in patients' tumor samples might predict the response to EO9.


http://www.sciencedirect.com/science/article/B6T4P-4DV1RN3-2/2/946352507c0bdbe2e695cde595211c73

Genistein has been reported to be a natural chemopreventive in several types of human cancer. In our prior study, soy isoflavones were shown to induce cell cycle arrest and apoptosis of bladder cancer cells in the range of human urine excretion. This study was designed to identify the novel molecular basis underlying anti-angiogenic activities of soy isoflavones. An immortalized E6 and five human bladder cancer cell lines were studied by immunoassay, flow cytometry, functional activity, reverse transcription-polymerase chain reaction, immunoblotting, and transwell co-culture in vitro. The efficacy of soy isoflavones on angiogenesis inhibition in vivo was examined by nude mice xenograft and chick chorioallantoic membrane bioassay. Factors analyzed included angiogenic factors, matrix-degrading enzymes, and angiogenesis inhibitors. Genistein was the most potent inhibitor of angiogenesis in vitro and in vivo among the isoflavone compounds tested. It may also account for most of the reduced microvessel density of xenografts observed and the suppressed endothelial migration by soy isoflavones. Genistein exhibited a dose-dependent inhibition of expression/excretion of vascular endothelial growth factor165, platelet-derived growth factor, tissue factor, urokinase plasminogen activator, and matrix metalloprotease-2 and 9, respectively. On the other hand, there was an up-regulation of angiogenesis inhibitors--plasminogen activator inhibitor-1, endostatin, angiotatin, and thrombospodin-1. In addition, a differential inhibitory effect between immortalized uroepithelial cells and most cancer cell lines was also observed. Altogether, we discovered that tissue factor, endostatin, and angiotatin are novel molecular targets of genistein. The current investigation provides further evidence in support of soy-based foods as natural dietary inhibitors of tumor angiogenesis.


http://www.sciencedirect.com/science/article/B6T4P-3VXH2BS-F/2/90122fb3086a4e1c1a4d59e960245f4d

Functional studies have shown that 6-chloro-9-[(3-methyl-2-butenyloxy]-3-methyl-1H-2,3,4, 5-tetrahydro-3-benzazepine (SKF 104078) has very low affinity for prejunctional [alpha]2-adrenoceptors ([alpha]2-AR) in the guinea pig atrium. In this study, we have cloned guinea pig homologues of the human [alpha]2-C10, [alpha]2-C2 and [alpha]2-C4 AR subtypes and have studied them in isolation by heterologous expression in cultured mammalian cells. Oligonucleotide primers, designed from conserved areas of the human [alpha]2-ARs were used in a polymerase chain reaction (PCR) with template cDNA synthesized from guinea pig atrial mRNA. Three PCR products were obtained that shared identity with the three human [alpha]2-AR subtypes. A guinea pig (gp) genomic library was screened with a cDNA clone encoding a portion of the gp-[alpha]2A, and genes containing the complete coding sequences of the guinea pig [alpha]2A, [alpha]2B, and [alpha]2C AR subtypes were obtained. These guinea pig genes were subcloned into a eukaryotic expression plasmid and were expressed transiently in COS-7 cells. The binding of the [alpha]2-selective antagonist [3H]MK-912 to membranes prepared from these cells was specific and of high affinity with Kd values of 810 pM for gp-[alpha]2A, 2700 pM for gp-
[alpha]2B and 110 pM for gp-[alpha]2C. Competition for the binding of [3H]MK-912 by SKF 104078 indicated that it was of moderately high affinity (~ 100 nM) but that it was not selective for any of the guinea pig [alpha]2-AR subtypes. Co-expression of guinea pig [alpha]2-AR subtypes with a cyclicAMP-responsive chloramphenicol acetyltransferase (CAT) reporter gene resulted in agonist-dependent modulation of CAT activity. For the gp-[alpha]2A, a biphasic response was obtained with low concentrations of noradrenaline (NE) decreasing forskolin-stimulated CAT activity and high concentrations causing a reversal. For the gp-[alpha]2B, NE produced mostly potentiation of forskolin-stimulated activity, and for the gp-[alpha]2C, NE caused mainly inhibition. Overall, the pharmacology of the cloned guinea pig [alpha]2-AR subtypes was in agreement with data obtained for the native guinea pig receptors and was functionally similar to that of the cloned human [alpha]2-AR subtypes.


http://www.sciencedirect.com/science/article/B6T4P-44RNPVM-N/2/0b57bbe531e9cece564be658dc5bc36f

We investigated the mitochondrial gene expression related to cardiac function and ventricular fibrillation (VF) in ischemic/reperfused nondiabetic and diabetic myocardium. To identify potentially more specific gene responses we performed subtractive screening, Northern blotting, and reverse transcription-polymerase chain reaction (RT-PCR) of mitochondrial genes expressed after 30 min ischemia followed by 120 min reperfusion in isolated rat hearts that showed VF or did not show VF. Cytochrome oxidase B subunit III (COXBIII) and ATP synthase subunit 6, studied and selected out of 40 mitochondrial genes by subtractive screening, showed an expression after 30 min ischemia (no VF was recorded) in both nondiabetic and diabetic subjects. Upon reperfusion, the down-regulation of these genes was only observed in fibrillated hearts. Such a reduction in signal intensity was not seen in nonfibrillated myocardium. In additional studies, nondiabetic and diabetic hearts, without the ischemia/reperfusion protocol, were subjected to electrical fibrillation, and a significant reduction in COXBIII and ATPS6 mRNA signal intensity was observed indicating that VF contributes to the down-regulation of these genes. Cardiac function (heart rate, coronary flow, aortic flow, left ventricular developed pressure) showed no correlation between the up- and down-regulation of these mitochondrial genes in both nondiabetic and diabetic ischemic/reperfused myocardium. Our data suggest that COXBIII and ATPS6 may play a critical role in arrhythmogenesis, and the stimulation of COXBIII and ATPS6 mRNA expression may prevent the development of VF in both nondiabetic and diabetic ischemic/reperfused myocardium.


http://www.sciencedirect.com/science/article/B6T4P-3V39SH9-72/2/d2dd22bfa266977d68543f5020dc232f

Agonist-mediated regulation of [beta]2-adrenoceptors in mononuclear leukocytes has been examined at the protein but not at the mRNA level. In the present study, incubation of mononuclear leukocytes with the [beta]-agonist (-)-isoproterenol (10-6 M) for up to 42 hr led to a maximum decrease in both [beta]2-adrenoceptor mRNA concentration and total receptor number of ca. 56 and 70%, respectively. The decrease in the mRNA level, however, was slower than for the protein level. After 4 hr of incubation with the [beta]-agonist, the protein level decreased to a minimum of 65% of the initial amount, while an incubation of 8 hr was necessary to reach a
similar decrease in the level of mRNA (69% of the initial level). Measurements of mRNA stability revealed a reduction in the half-life of \([\beta]2\)-adrenoceptor mRNA from 2.7 to 1.1 hr following 4 hr of incubation with (-)-isoproterenol. Our data clearly demonstrate that treatment of human mononuclear leukocytes with (-)-isoproterenol induces a \([\beta]2\)-adrenoceptor down-regulation together with a slower time course of mRNA down-regulation which is partly due to a reduction of mRNA stability.


Sister of P-glycoprotein (spgp) is a gene that is closely related to the P-glycoprotein family (Pgps). This class of proteins belongs to the superfamily of ATP-binding cassette transporters and is known for its involvement in pharmacological drug interactions. Therefore, this study investigated the distribution of spgp expression in different tissues known for their high levels of Pgps expression such as brain, liver, kidney, small- and large-gut mucosa. Analysis was done by using the reverse transcription-polymerase chain reaction. In addition to a high expression in the liver, we were able to demonstrate a significant spgp expression in brain grey cortex, small- and large-gut mucosa. Although Pgps are expressed in the kidney and brain capillary endothelial cells, no expression of spgp was detected in these tissues, which might indicate that spgp has no function in the blood-brain barrier and is not involved in the renal excretion of drugs.


3'-Phosphoadenosine 5'-phosphosulfate (PAPS) is the high-energy "sulfate donor" for reactions catalyzed by sulfotransferase (SULT) enzymes. The strict requirement of SULTs for PAPS suggests that PAPS synthesis might influence the rate of sulfate conjugation. In humans, PAPS is synthesized from ATP and SO4^2- by two isoforms of PAPS synthetase (PAPSS): PAPSS1 and PAPSS2. As a step toward pharmacogenetic studies, we have resequenced the entire coding sequence of the human PAPSS1 gene, including exon-intron splice junctions, using DNA samples from 60 Caucasian-American and 58 African-American subjects. Twenty-one genetic polymorphisms were observed--1 insertion-deletion event and 20 single nucleotide polymorphisms (SNPs)--including two non-synonymous coding SNPs (cSNPs) that altered the following amino acids: Arg333Cys and Glu531Gln. Twelve pairs of these polymorphisms were tightly linked, and a total of twelve unequivocal haplotypes could be identified--two that were common to both ethnic groups and ten that were ethnic-specific. The Arg333Cys polymorphism, with an allele frequency of 2.5%, was observed only in DNA samples from Caucasian subjects. The Glu531Gln polymorphism was rare, with only a single copy of that allele in a DNA sample from an African-American subject. Transient expression in mammalian cells showed that neither of the non-synonymous cSNPs resulted in a change in the basal level of enzyme activity measured under optimal assay conditions. However, the Glu531Gln polymorphism altered the substrate kinetic properties of the enzyme. The Gln531 variant allozyme had a 5-fold higher Km value for SO4^2- than did the wild-type allozyme and displayed monophasic kinetics for Na2SO4. The wild-type allozyme (Glu531) showed biphasic kinetics for that substrate. These observations represent a step toward testing the hypothesis that genetic variation in PAPS synthesis catalyzed
by PAPSS1 might alter in vivo sulfate conjugation.

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http://www.sciencedirect.com/science/article/B6T4R-3YCMKSW-28/2/7b9bcde715818c040e3fa48be11f5483

Two congeneric species of brackish water clams, Rangia cuneata (Sowerby, 1831) and Rangia flexuosa (Conrad, 1839), that are sympatric in the northern Gulf of Mexico, were analyzed for electrophoretic a variation at 19 allozyme loci. Rangia cuneata also was analyzed for restriction-site and (partial) nucleotide sequence variation for a mitochondrial gene, cytochrome oxidase I (COI). Rangia cuneata has greatly increased its abundance along the mid-Atlantic coast of the U.S.A. within the last 100 years, due either to colonization from southern populations or to expansion of indigenous populations. Rangia cuneata populations from Virginia to southern Florida formed a discrete and homogeneous group based on allozyme allele frequencies, as did populations from Mississippi to Texas (Nei's unbiased genetic distance between the two groups was 0.03). Atlantic coast populations of R. cuneata also had uniformly lower expected heterozygosities and lower numbers of alleles per locus than Gulf populations. The COI gene was largely monomorphic and mostly uninformative about possible genetic differences between Atlantic and Gulf coast R. cuneata, but a polymorphic MboII restriction site in the Gulf was monomorphic in all examined Atlantic populations. These data are more consistent with the indigenous-expansion model than the southern-expansion model. Nei's genetic distance between R. cuneata and R. flexuosa was large (1.45), but there was no evidence for the existence of a complex of sibling or semi-species within R. cuneata.


http://www.sciencedirect.com/science/article/B6T4R-47S10PH-3/2/45001087a12b8ae78341c03a554fa7da

We analyzed sequence data from 107 base pairs within the nuclear 18S ribosomal RNA gene from 26 tephritid species and four out-group taxa. A total of 45 sites were variable within the Tephritidae, and 28 sites were considered informative for parsimony analysis. Phylogenetic information was extracted from this data set using maximum parsimony and neighbor joining methods and compared to a phylogenetic hypothesis proposed from the morphological literature. Our molecular data suggest: (1) monophyly of the subfamily Tephritinae; (2) membership of Gymnocarena; (3) the possible existence of a large monophyletic group including the subfamily Dacinae and a large part of the Trypetinae. We propose that molecular approaches show great promise for helping to create a phylogenetically-based higher classification for the Tephritidae.

http://www.sciencedirect.com/science/article/B6T4R-447NKGM-5/2/699e8ecbada9b31283b7a5ac8cb92c4e

Random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity in three species of toxic larkspurs (Delphinium spp). A total of 184 plants from 22 accessions in five western states were analyzed by 23 RAPD primers that amplified 188 reproducible bands. There were 144 polymorphic bands; 10 shared by Delphinium glaucum and Delphinium occidentale, eight shared by Delphinium barbeyi and D. glaucum, and 18 shared by D. occidentale and D. barbeyi. Thirteen bands were specific for D. occidentale, 18 for D. glaucum and 19 for D. barbeyi. There were 58 bands that were specific for individual accessions and 44 bands that were common to all three species. Some of the species-specific bands were cloned and tested in Southern hybridization. Based on the presence or absence of the 144 polymorphic RAPD bands in individuals, a dendrogram was generated to assess the genetic similarity among the samples. The cophenetic values were 0.64 between D. occidentale and D. barbeyi, and 0.55 between the cluster of these two species and D. glaucum. These relationships are congruent with those based on morphological characters and support the contention that these are separate species. Understanding the genetic relationships among these three tall larkspur species will provide basic knowledge useful in developing strategies to reduce livestock losses by these poisonous plants.


We report on the identification of unique sequences within the highly variable rDNA ITS regions and more conserved 5.8S regions which provide data for the design of species- and genus-specific probes, and we examine some aspects of the phylogeny of Alexandrium isolates from New Zealand waters.


http://www.sciencedirect.com/science/article/B6T4R-3W4930J-6/2/4937f24e4e0d62055a191c4a07e848c4

The systematic relationships of the Class Raphidophyceae within the chromophyte algae are uncertain. The 'V4' domain small-subunit rDNA gene ('V4' domain SSU rDNA gene) was sequenced for the taxa Heterosigma carterae and Chattonella antiqua to provide characters for phylogenetic analyses. These analyses showed that the Classes Raphidophyceae and Eustigmatophyceae form a sister clade. A close systematic relationship for these two classes has not been shown before. Numerous phylogenetic analyses in this study have failed to provide resolution of the deep branches of the five major evolutionary assemblages--Classes Raphidophyceae and Eustigmatophyceae; Classes Synurophyceae and Chrysophyceae; Classes Phaeophyceae and Xanthophyceae; Class Bacillariophyceae; Class Prymnesiophyceae. Transition frequency analysis shows that there is a major loss of phylogenetic signal within the
chromophyte algae V4 domain SSU rDNA gene sequences.


http://www.sciencedirect.com/science/article/B6T4R-3YVDB0Y-4/2/958a6a2dd2d9c48c4295d6af0e6412e

DNA sequence and morphological data were analyzed for specimens of twenty-five species of acanthomorph fishes and two specimens representing the outgroups Aulopiformes and Myctophiformes. A 572 base-pair (bp) segment of the 12S ribosomal mitochondrial gene, 1112 bp from three regions of the 28S ribosomal nuclear gene, and 38 morphological transformation series were analyzed under the criterion of maximum parsimony. The total evidence analysis resulted in a set of four most parsimonious trees. Relationships common to all trees are largely congruent with the hypothesis articulated by Johnson and Patterson (1993. Bull. Mar. Sci. 52, 554-626).

Biochimica et Biophysica Acta (BBA) - Bioenergetics(2)


http://www.sciencedirect.com/science/article/B6T1S-3XY2R40-V/2/8efad893aa9330cd634ab7c0f975ef80f

Recent studies have shown that the NADPH oxidase participates in the generation of superoxide anion in non-phagocytic cells. Here we report the isolation and nucleotide sequence of a cDNA for the cytochrome b-558 (alpha)-subunit of the NADPH oxidase in rat vascular smooth muscle cells (VSMCs). The coding region of the cDNA was 93% homologous to mouse and 81% to human in nucleotide sequence and 96% homologous to mouse and 89% to human in the deduced amino acid sequence. Our results provide a tool with which to explore the mechanism of superoxide anion generation in rat VSMCs and other non-phagocytic cells.


http://www.sciencedirect.com/science/article/B6T1S-3X88CMS-6/2/abf193d42329a999f430f975e23267f5

Uncoupling protein 1 (UCP1) is of demonstrated importance in mammalian thermogenesis, and early hypotheses regarding the functions of the newly discovered UCP homologues, UCP2, UCP3 and others, have focused largely on their potential roles in thermogenesis. Here we report the amino acid sequences of two new UCPs from ectothermic vertebrates. UCPs from two fish species, the zebrafish (Danio rerio) and carp (Cyprinus carpio), were identified in expressed
sequence tag databases at the European Molecular Biology Laboratory. cDNAs from a C. carpio 'peritoneal exudate cell' cDNA library and from a D. rerio 'day 0 fin regeneration' cDNA library were obtained and fully sequenced. Each cDNA encodes a 310 amino acid protein with an average 82% sequence identity to mammalian UCP2s. The fish UCP2s are about 70% identical to mammalian UCP3s, and 60% identical to mammalian UCP1s. Carp and zebrafish are ectotherms - they do not raise their body temperatures above ambient by producing excess heat. The presence of UCP2 in these fish thus suggests the protein may have function(s) not related to thermogenesis.

Biochimica et Biophysica Acta (BBA) - Biomembranes (8)


http://www.sciencedirect.com/science/article/B6T1T-47MHVW8-1/2/ad5df9df9889b3031177ea54c342aae8

The aim of this work is to develop a prokaryotic system capable of expressing membrane-bound receptors in quantities suitable for biochemical and biophysical studies. Our strategy exploits the endogenous high-level expression of the membrane protein bacteriorhodopsin (BR) in the Archaeon Halobacterium salinarum. We attempted to express the human muscarinic acetylcholine (M1) and adrenergic (a2b) receptors by fusing the coding region of the m1 and a2b genes to nucleotide sequences known to direct bacterio-opsin (bop) gene transcription. The fusions included downstream modifications to produce non-native carboxyl-terminal amino acids useful for protein identification and purification. bop mRNA and BR accumulation were found to be tightly coupled and the carboxyl-terminal coding region modifications perturbed both. m1 and a2b mRNA levels were low, and accumulation was sensitive to both the extent of the bop gene fusion and the specific carboxyl-terminal coding sequence modifications included. Functional a2b adrenergic receptor expression was observed to be dependent on the downstream coding region. This work demonstrates that a critical determinant of expression resides in the downstream coding region of the wild-type bop gene and manipulation of the downstream coding region of heterologous genes may affect their potential for expression in H. salinarum.


http://www.sciencedirect.com/science/article/B6T1T-482YJ0R-2/2/7d813b567b42c3afe1ee5c053af4b5b1

We investigated the influence of intracellular pH (pHi) on [14C]-glycocholate (GC) uptake by human hepatoblastoma HepG2 cells that express sodium-independent (mainly OATP-A and OATP-8), but not sodium-dependent, GC transporters. Replacement of extracellular sodium by choline (Chol) stimulated GC uptake but did not affect GC efflux from loaded cells. Amiloride or NaCl replacement by tetraethylammonium chloride (TeACl) or sucrose also increased GC uptake. All stimulating circumstances decreased pH. By contrast, adding to the medium ammonium or
imidazole, which increased pH, had no effect on GC uptake. In Chinese hamster ovary (CHO) cells expressing rat Oatp1, acidification of pH had the opposite effect on GC uptake, that is, this was reduced. Changes in extracellular pH (pHo) between 7.40 and 7.00 had no effect on GC uptake at pH 7.30 or 7.45 when pHo-pHi. Inhibition was not proportional to the pHo-pHi difference. Intracellular acidification decreased Vmax, but had no effect on Km. In sum, sodium-independent GC transport can be affected by intracellular acidification, possibly due both to modifications in the driving forces and to the particular response to protonation of carrier proteins involved in this process.


http://www.sciencedirect.com/science/article/B6T1T-3W7XB4D-V/2/95a17135ef17cb84c138f62ebeb2c316

We have cloned a cDNA for vacuolar proton-translocating pyrophosphatase of Chara corallina that is one of the closest green algae to the land plants. The deduced protein consists of 793 amino acid residues. Its sequence is 71% identical to the H+-pyrophosphatases of land plants, and is less than 46% identical to those of marine alga and phototrophic bacterium.


http://www.sciencedirect.com/science/article/B6T1T-4CX6SD7-1/2/7a298c5d9d3ed29758565ac50c3dbff6

In this work, we studied the mRNA distribution of CNG-A3, an amiloride-sensitive sodium channel that belongs to the cyclic nucleotide-gated (CNG) family of channels, along the rat nephron. The possible involvement of aldosterone in this process was also studied. We also evaluated its expression in rats subjected to diets with different concentrations of sodium or to alterations in aldosterone plasma levels. Total RNA isolated from whole kidney and/or dissected nephron segments of Wistar rats subjected to low- and high-sodium diets, furosemide treatment, adrenalectomy, and adrenalectomy with replacement by aldosterone were analyzed by the use of Western blot, ribonuclease protection assay (RPA) and/or reverse transcription followed by semi-quantitative polymerase chain reaction (RT-PCR). CNG-A3 sodium channel mRNA and protein expression, in whole kidneys of rats subjected to high-Na+ diet, were lower than those in animals given a low-salt diet. Renal CNG-A3 mRNA expression was also decreased in adrenalectomized rats, and was normalized by aldosterone replacement. Moreover, a CNG-A3 mRNA expression study in different nephron segments revealed that aldosterone modulation is present in the cortical thick ascending loop (cTAL) and cortical collecting duct (CCD). This result suggests that CNG-A3 is responsive to the same hormone signaling as the amiloride sensitive sodium channel ENaC and suggests the CNG-A3 may have a physiological role in sodium reabsorption.

The Na+/dicarboxylate cotransporter, NaDC-1, and the Na+/sulfate cotransporter, NaSi-1, share 43% sequence identity, but they exhibit no overlap in substrate specificity. A functional chimera, SiDC-4, was prepared from NaDC-1 and NaSi-1 by homologous recombination and expressed in Xenopus oocytes. SiDC-4 contains putative transmembrane domains 1-4 of NaSi-1 (amino acids 1-139) and putative transmembrane domains 5-11 of NaDC-1 (amino acids 141-593). SiDC-4 retains the substrate specificity of NaDC-1, which suggests that the substrate recognition domain is found in the carboxy-terminal portion of the protein, past amino acid 141. However, residues that affect substrate affinity and inhibition by furosemide and flufenamate are found in the amino terminal third of the protein. The cation binding properties of SiDC-4, including a stimulation of transport by lithium, differed from both parental transporters, suggesting that cation binding is determined by interactions between the amino- and carboxy-terminal portions of the protein. We conclude that the substrate recognition site of NaDC-1 and NaSi-1 is found in the carboxy-terminal portion of the protein, past amino acid 141, but residues in the amino terminus can affect substrate affinity, inhibitor sensitivity, and cation selectivity.


Background and Aims This study aimed at functional characterization of the tight junction protein occludin using the occludin-deficient mouse model. Methods Epithelial transport and barrier functions were characterized in Ussing chambers. Impedance analysis revealed the ionic permeability of the epithelium (Re, epithelial resistance). Conductance scanning differentiated transcellular (Gc) and tight junctional conductance (Gtj). The pH-stat technique quantified gastric acid secretion. Results In occludin+/+ mice, Re was 23±5 Ω cm² in jejunum, 66±8 Ω cm² in distal colon and 33±6 Ω cm² in gastric corpus. Re was not altered in heterozygotic occludin+/- or homozygotic occludin-/- mice. Additionally, [3H]mannitol fluxes were unaltered. In the control colon, Gc and Gtj were 7.6±0.8 Ω cm² and 0.3±0.1 Ω cm², respectively. In occludin deficiency, epithelial resistance after mechanical perturbation or EGTA exposition (low calcium switch) was not more affected in occludin-/- mice than in control. Barrier function was measured in the urinary bladder, a tight epithelium, and in the stomach. Control Rt was 5.9±0.4 Ω cm² in urinary bladder and 33±6 Ω cm² in stomach and not altered in occludin-/- mice. In gastric corpus mucosa, the glandular structure exhibited a complete loss of parietal cells and mucus cell hyperplasia, as a result of which acid secretion was virtually abolished in occludin-/- mice. Conclusion Epithelial barrier characterization in occludin-deficiency points against an essential barrier function of occludin within the tight junction strands or to a substitutional redundancy of single tight junction molecules like occludin. A dramatic change in gastric morphology and secretory function indicates that occludin is involved in gastric epithelial differentiation.


http://www.sciencedirect.com/science/article/B6T1T-47GGV6D-8Y/2/d1b7f11f3e1580b656e94976cca20622
Taurine was shown recently to increase the frequency at which 2-cell mouse conceptuses develop into blastocysts in vitro. For this reason and because taurine helps cells adapt to external stresses, we studied transport of this and related amino acids by preimplantation mouse conceptuses. The most conspicuous component of taurine transport in conceptuses at the 1-cell through blastocyst stages of development was both Na+- and Cl--dependent. This Na+- and Cl--dependent transport system interacted relatively strongly with [beta]- but not [alpha]-amino acids. By these criteria, transport system [beta] is responsible for Na+-dependent taurine transport in preimplantation mouse conceptuses. Moreover, detection of mRNA encoding the taurine transport protein (TAUT) in early conceptuses supports the theory that TAUT is a major component of system [beta]. Transport of taurine by system [beta] in 1-cell conceptuses was slower in hypotonic than in hypertonic medium, whereas the reverse was true for system [beta] in blastocysts. In contrast, hypotonically stimulated Na+-independent taurine transport was, of course, more rapid in hypotonic than in hypertonic medium in both 1-cell conceptuses and blastocysts. Transport via this hypotonically stimulated process also showed no sign of saturation by up to 10 mM taurine. Hypotonically stimulated taurine transport appeared transiently in 1-cell conceptuses under hypotonic conditions until they had recovered their initial volumes. Hence, we suggest that a decrease in taurine uptake via system [beta] and an increase in taurine exodus via the Na+-independent, nonsaturable transport process could contribute to the regulatory volume decrease in 1-cell conceptuses in hypotonic medium. Since taurine uptake by system [beta] in blastocysts is, however, higher in hypotonic than in hypertonic medium, taurine uptake by system [beta] in blastocysts might intensify a tendency to increase cell volume in hypotonic medium. Such an increase in taurine uptake could further favor anabolic changes associated with cell swelling. In addition to contributing to regulation of cellular volume and perhaps metabolism, the hypotonically stimulated Na+-independent transport processes in early embryos have novel characteristics. Hypotonically stimulated Na+-independent taurine transport was inhibited by niflumate, N-ethylmaleimide and NaN3 but not by furosemide, iodoacetate, KCN, ouabain or [alpha]- or [beta]-amino acids. Furthermore, 4,4’-disothiocyanostilbene-2,2’-disulfonate inhibited this transport in 1-cell conceptuses but not in blastocysts. Hence, different hypotonically stimulated Na+-independent taurine transport processes appear to be present in 1-cell conceptuses vs. blastocysts. The functions of these and other instances of developmental regulation of expression of transport processes in preimplantation conceptuses remain largely to be elucidated. Moreover, neither of the hypotonically stimulated Na+-independent taurine transport processes in conceptuses appears to have been detected in other types of cells. Instead, these processes may be unique to preimplantation conceptuses.


http://www.sciencedirect.com/science/article/B6T1T-3V3RHKC-1F/2/205e3de09ca4b5d87f9f957ace7023940

Recent evidence that insulin-like growth factor-1 (IGF-1) influences certain properties of H4IIE hepatoma cells independent of insulin led us to examine whether H4IIE cells express IGF-1 receptors. Competitive binding experiments demonstrated IGF-1, but not insulin or IGF-II, could compete with [125I]IGF-1. Chemical crosslinking detected a protein with an apparent mass of 175 kDa and its identity as the IGF-1 receptor [alpha]-subunit was confirmed by Western blotting. The apparent molecular mass of this protein decreased to 135 kDa following deglycosylation. Immunofluorescence microscopy verified that both insulin and IGF-1 receptors were present, although measurement of IGF-1 receptor quantity revealed they were less abundant than insulin receptors. Binding of IGF-1 was low in growing cells and higher in a quiescent cell population. Scatchard analysis confirmed that receptor density was increased in non-growing H4IIE cells while there was no apparent difference in receptor affinity. Western blot analysis and RT-PCR revealed that both protein and mRNA levels were elevated as cell growth ceased. Interestingly,
addition of insulin to quiescent H4IIE cells, which stimulates cell proliferation, further increased IGF-1 receptor protein levels with a peak at 12-24 h. Distinct modes of regulating IGF-1 receptor expression are indicated.

Biochimica et Biophysica Acta (BBA) - General Subjects  (16)


http://www.sciencedirect.com/science/article/B6T1W-42VV80F-5/2/7f2f4faffbcfc140f26c806069b97c18

The aim of this study was to characterize the cellular phenotypes of articular cartilage and meniscus in rabbits with experimentally induced osteoarthritis (OA), by histological and molecular biological techniques. OA was induced by severing the anterior cruciate ligament of the knee and rabbits were killed 2, 4 or 9 weeks following surgery. Our histological observations show a progressive destruction of extracellular matrix in both tissues. To determine whether these morphological changes could be related to alterations in the regulation of gene expression for a subset of relevant molecules, levels of mRNA for proteinases and one inhibitor (MMP-1, -3 and -13, aggrecanase-1 and -2 and TIMP-1), matrix molecules and one chaperone (type II and X collagens, aggrecan, osteonectin, [beta]ig-h3 and BiP) were assessed by reverse transcription-polymerase chain reaction. Our results indicate that for most markers expression profiles were similar in both tissues. In particular, matrix protein gene expression remained stable or varied little during progression of OA, suggesting a poor repair capacity of the tissues. MMP gene expression increased rapidly whereas aggrecanase gene expression remained stable. These findings suggest that differential regulation of mRNA levels of MMP-1, -3 and -13 on the one hand and aggrecanase-1 and -2 on the other, occurs during OA.


http://www.sciencedirect.com/science/article/B6T1W-3YN9Z7B-H/2/3bf3435a4aed8f0e42ba8e6c160b9f13

Colonization of the human stomach by Helicobacter pylori is associated with the development of gastritis, duodenal ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. H. pylori-antigen-binding single-chain variable fragments (ScFv) were derived from murine hybridomas producing monoclonal antibodies and expressed as a g3p-fusion protein on a filamentous M13 phage. The recombinant ScFv-phage reacted specifically with a 30-kDa monomeric protein of a H. pylori surface antigen preparation and by means of immunofluorescence microscopy the phage was shown to bind to both the spiral and coccolid forms of the bacterium. In vitro, the recombinant phage exhibited a bacteriocidal effect and inhibited specifically the growth of all the six strains of H. pylori tested. When H. pylori was pretreated with the phage 10 min before oral inoculation of mice, the colonization of the mouse stomachs by the bacterium was significantly reduced (P<0.01). The results suggest that genetic
engineering may be used to generate therapy-effective phages.


http://www.sciencedirect.com/science/article/B6T1W-49031HB-2/2/119cc7ca6fe06a0f96356f2abc10ddb0

Rodent cells, widely used for the industrial production of recombinant human glycoproteins, possess CMP-N-acetylneuraminic acid hydroxylase (CMP-Neu5Ac hydroxylase; EC 1.14.13.45) which is the key enzyme in the formation of the sialic acid, N-glycolylneuraminic acid (Neu5Gc). This enzyme is not expressed in an active form in man and evidence suggests that the presence of Neu5Gc in recombinant therapeutic glycoproteins may elicit an immune response. The aim of this work was, therefore, to reduce CMP-Neu5Ac hydroxylase activity in a Chinese Hamster Ovary (CHO) cell line, and thus the Neu5Gc content of the resulting glycoconjugates, using a rational antisense RNA approach. For this purpose, the cDNA of the hamster hydroxylase was partially cloned and sequenced. Based on the sequence of the mouse and hamster cDNAs, optimal antisense RNA fragments were selected from preliminary in vitro translation tests. Compared to the parental cell line, the new strain (CHO-AsUH2), which was transfected with a 199-bp antisense fragment derived from the mouse CMP-Neu5Ac hydroxylase cDNA, showed an 80% reduction in hydroxylase activity. An analysis of the sialic acids present in the cells' own glycoconjugates revealed a decrease in the percentage of Neu5Gc residues from 4% in the parental cells to less than 1% in the CHO-AsUH2 cell line.


http://www.sciencedirect.com/science/article/B6T1W-49W63M1-1/2/f88e7f3c16fc7281dc0f50f402d9450

We investigated the efficiency and the mechanism of action of a tetraphenyl porphyrin derivative in its photoreaction with T7 phage as surrogate of non-enveloped DNA viruses. TPFP was able to sensitize the photoinactivation of T7 phage in spite of the lack of its binding to the nucleoprotein complex. The efficiency of TPFP photosensitization was limited by the aggregation and by the photobleaching of porphyrin molecules. Addition of sodium azide or 1,3-dimethyl-2-thiourea (DMTU) to the reaction mixture moderated T7 inactivation, however, neither of them inhibited T7 inactivation completely. This result suggests that both Type I and Type II reaction play a role in the virus inactivation. Optical melting studies revealed structural changes in the protein part but not in the DNA of the photochemically treated nucleoprotein complex. Polymerase chain reaction (PCR) also failed to demonstrate any DNA damage. Circular dichroism (CD) spectra of photosensitized nucleoprotein complex indicated changes in the secondary structure of both the DNA and proteins. We suggest that damages in the protein capsid and/or loosening of protein-DNA interaction can be responsible for the photodynamic inactivation of T7 phage. The alterations in DNA secondary structure might be the result of photochemical damage in phage capsid proteins.

P450 genes and their negative regulation by RE1 silencing transcription factor/neuron-restrictive silencer factor." Biochimica et Biophysica Acta (BBA) - General Subjects 1620(1-3): 39.

http://www.sciencedirect.com/science/article/B6T1W-47C4CJV-4/2/ec9cfdd03b0bf63e0bcf2fcb32388907

RE1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) mediates transcriptional repression in many neuron-specific genes by interaction with the repressor element 1/neuron-restrictive silencing element (RE1/NRSE). This element has been identified at least in 20 neuron specific genes. REST/NRSF is highly expressed in non-neuronal tissues, where it is thought to repress gene transcription. We performed a BLAST search to look for the presence of RE1/NRSE elements in the rat cytochrome P450 genes. We identified the presence of RE1/NRSE element in the cytochrome P450 genes CYP1A1, 2A2, 2E1 and 3A2. Electrophoretic mobility shift assay and supershift assays were carried out to prove functionality of these sites and detect the interaction of REST/NRSF with this sequence. Cotransfection studies in PC12 cells with a plasmid containing the RE1 element of the CYP genes, cloned upstream of the minimal type II sodium channel promoter, in the presence of REST/NRSF, showed a marked expression inhibition of the CAT reporter gene. These data suggest that the RE1 elements that exist in these four CYP genes might be a target for the REST/NRSF transcription factor and such an interaction might play a role in the negative regulation of these genes.


http://www.sciencedirect.com/science/article/B6T1W-477GHXS-2/2/b4a0b41d053c31f9866cf2e50c43abae

The present study constitutes the first finding of the calcium-binding protein S100B and of its mRNA in human milk, as revealed by a quantitative immunoluminometric assay, by Western blot analysis and by reverse transcription-polymerase chain reaction (RT-PCR) assay followed by restriction enzyme digestion. The concentration of S100B in milk is markedly higher than that observed in other biological fluids such as cord blood, peripheral blood, urine, cerebrospinal fluid and amniotic fluid. This finding could be related to a possible trophic role, which has been hypothesized for the protein.


http://www.sciencedirect.com/science/article/B6T1W-42VV80F-D/2/f74215836b9cbe2d6c89ff3148fccc84

Lsh is a member of the SNF2 family of chromatin remodelers, that regulate diverse biological processes such as replication, repair and transcription. Although expression of Lsh is highly tissue specific in adult animals, Lsh mRNA is detectable in multiple tissues during embryogenesis. In order to determine the physiologic role of Lsh during murine development and to assess its unique function in adult mice, we performed targeted deletion of the Lsh gene using homologous recombination in murine embryonic stem cells. Lsh-/- embryos occurred with the expected Mendelian frequency after implantation and during embryogenesis. However, Lsh-/- mice died within a few hours after birth. Furthermore, newborn mice were 22% lower in weight in comparison with their littersmates and showed renal lesions. Thus Lsh is a non-redundant member
of the SNF2 family and is essential for normal murine development and survival.


http://www.sciencedirect.com/science/article/B6T1W-47MJH0R-15W/2/d44eec10735d99a84686b1af1c406add

Cellular retinol-binding protein II (CRBP II) is an abundant cytosolic protein of intestinal absorptive cells. In this study, we examined whether dietary fat modulates the expression of CRBP II in the small intestine. In the rats fed a diet rich in long-chain triacylglycerols (LCT), both CRBP II mRNA and CRBP II protein levels in the jejunum were more than two-fold greater than in the rats fed a low fat diet and a diet rich in medium-chain triacylglycerols (MCT). The mRNA abundance of a retinoid X receptor (RXR[alpha]), which is thought to interact with the cis-element located in the CRBP II promoter, was elevated in the jejunum of rats fed high-LCT and high-MCT diets as compared with that of animals fed a low-fat diet, but the levels of RXP[alpha] mRNA of the LCT diet groups was similar to that of MCT diet group. These results suggest that the expression level of the CRBP II gene is not directly related to the RXR[alpha] expression, and that it might be modulated by long-chain fatty acids or their metabolites.


http://www.sciencedirect.com/science/article/B6T1W-48GVKWS-1/2/c52d1dbcede58ab339e8ddcac10a36b2

For an understanding of tumor-related alterations of the complex carbohydrate pattern of carcinomas, it is indispensable to monitor the expression profile of the various glycosyltransferases. The objective of this contribution was to perform an evaluation of the usefulness and the limits of the microarray approach for the identification of enzymes responsible for carbohydrate synthesis with differential expression in carcinomas. Expression profiles of colonic carcinomas were studied by oligonucleotide arrays using a novel strategy: colonic tissue of healthy individuals was compared with early staged colonic carcinomas; 'pure' cell populations were obtained by laser microdissection; RNA samples for hybridization with the oligonucleotide arrays were prepared by in vitro transcription without additional amplification. Expression of 39 glycosyltransferases and of 10 sulfotransferases in colonic tissues was analyzed by Affymetrix GeneChip technology. GeneChip analysis proved the high expression level of ST6Gal-I, [beta]4Gal-TI, II and III, GalNacT-1, FT-III and showed that ST3Gal-IV was the most abundantly expressed enzyme in healthy tissue. The strong overexpression of FT-VI in healthy tissue has not been described so far, as well as the upregulation of FT-VIII and downregulation of GnT-I in carcinoma tissue. Quantitative RT-PCR confirmed that FT-VI expression was significantly enhanced in healthy tissue. On the other hand, GeneChip analysis failed to detect any expression of GnT-III and GnT-V as well as of ST3Gal-I and ST3Gal-II, although these sequences could be amplified from the samples used for microarray analysis. According to our restricted analysis of only those 39 glycosyltransferases present on the GeneChip U95A, alterations of sialyltransferases ST6Gal-I, ST3Gal-IV, of fucosyltransferases FT-VI, FT-III, and probably FT-VIII, of GalNacT-1, and of [beta]4GalT-II seem to be of relevance for the aberrant biosynthesis of membrane-bound carbohydrates during colonic carcinogenesis and metastasis.

http://www.sciencedirect.com/science/article/B6T1W-3R38XW3-N/2/2536d759198610b95cd258fdeb28fbe2

Since there are conflict reports on the presence of -amino-acid oxidase in the mouse liver, this problem was examined. -Amino-acid oxidase activity was not detected in the homogenates of the mouse liver, lung, or heart, whereas it was detected in the homogenates of the mouse kidney and brain. Western blotting showed that a protein which reacted with the antiserum against pig -amino-acid oxidase was present in the homogenates of the mouse kidney and brain but not in those of the liver or heart. Northern hybridization using a -amino-acid oxidase cDNA probe detected a hybridizing signal in poly(A)+ RNAs extracted from the mouse kidney and brain but not in those from the liver, heart, or lung. Reverse transcription-polymerase chain reaction using three primer pairs always amplified -amino-acid oxidase cDNA fragments of expected sizes in the mouse kidney and brain but very rarely did so in the liver, heart, or lung. The results indicate that -amino-acid oxidase is not present in the mouse liver in a measurable amount.


http://www.sciencedirect.com/science/article/B6T1W-4F7HH68-2/2/0b85f450475f0062a8784d4f20885ccc3

We cloned the feruloyl esterase A gene from Aspergillus awamori (AwfaeA) and engineered it to study substrate specificity and pH dependence of catalysis. Based on the crystal structures of two type-A feruloyl esterases (FAE-III and AnFAEA) from Aspergillus niger, residues located in the flap region of AwFAEA (Asp71, Thr72, Asp77, and Tyr80) were replaced with corresponding amino acid residues (Ile, Arg, Asn, and Phe), respectively, found in the lid of lipases from Rhizomucor miehei (RmLIP) and Humicola lanuginose (HILIP). Furthermore, Asp77 of AwFAEA, which is conserved in Aspergillus FAEs and lipases, was replaced with a hydrophobic residue (Ile). Kinetic analysis of the mutant enzymes showed that the higher catalytic efficiency of the D77I and Y80F mutants toward [alpha]-naphthylbutyrate (C4) and [alpha]-naphthylcaprylate (C8), respectively, was due to a lower Km value. The higher catalytic efficiency of D77N toward C4 substrate was due to a combination of decreased Km and considerably increased kcat. The D77I and Y80F mutants showed some activity toward long-acyl chain esters. On the other hand, the D77I mutant had no detectable activity toward phenolic acid methyl esters and feruloylated arabinoxylan. Moreover, the pH optima of the D77I, D77N, and Y80F mutants increased from 5.0 to 7.0-8.0, 7.0, and 6.0, respectively.


http://www.sciencedirect.com/science/article/B6T1W-48KW78P-2/2/b6f60b7e69a93f6d43c471a112da4bffe7

Hemoglobins (Hbs) are heme proteins encountered in all five kingdoms of living organisms. In plants, two different classes of Hbs have been identified: nonsymbiotic (class I) from both monocot and dicot species and symbiotic (class II) Hbs from nitrogen-fixing plants. This work
reports the cloning and analysis of three nonsymbiotic Hb genes from wheat (Triticum aestivum) and potato (Solanum tuberosum). The Hb cDNAs were amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using consensus oligonucleotide primers for nonsymbiotic Hbs. A wheat Hb cDNA (TaHb1) was isolated and shows a very high similarity to nonsymbiotic Hbs from Hordeum vulgare (98%) and Zea mays (83%). Another wheat Hb cDNA, designated TaHb2, exhibited strong similarity to truncated bacterial Hbs, the so-called 2-on-2 Hbs. In addition, a third Hb was cloned from potato, StHb. Expression analysis by RT-PCR demonstrated a very high expression level of the TaHb1 gene only in wheat roots. In contrast, the other wheat hemoglobin gene, TaHb2, was demonstrated to be constitutively expressed although differences in expression level in different tissues were observed. The expression of the TaHb1 gene is induced in wheat roots exposed to microaerobic conditions. The potato Hb gene, StHb, was highly expressed in roots and also in tubers and stem tissue although at much reduced levels.


http://www.sciencedirect.com/science/article/B6T1W-3T8WG4K-4/2/47babbc54137d53ba43ad195caf2f6ec

Single-chain antibody fragments (scAbs), which have a human C-kappa constant domain and a hexa-histidine tail attached to the carboxy terminus of the single-chain Fv (ScFv) fragments to facilitate purification, have been raised against the herbicides paraquat and atrazine and expressed in transgenic Nicotiana tabacum cv. Samsun NN. Prior to purification, the anti-atrazine scAb is expressed as up to 0.014% of soluble leaf protein and has a binding profile in ELISA, against an atrazine-bovine serum albumin (BSA) conjugate, similar to that of the scAb produced in Escherichia coli. Competition ELISA has shown that the plant-derived scAb also recognises free atrazine. Following antibody affinity purification to isolate dimers, the affinity for immobilised antigen approaches that of the parental monoclonal antibody. This was confirmed by surface plasmon resonance analysis. The purified scAb also recognises related triazine herbicides. When isolated from cell-suspension cultures, the anti-paraquat scAb binds to a paraquat conjugate in a concentration-dependent manner, with a profile similar to the parental monoclonal antibody. This is the first demonstration that functional scAbs against organic pollutants can be produced in transgenic plants and that the scAbs may be appropriate for the development of immunoassay-based detection systems.


http://www.sciencedirect.com/science/article/B6T1W-3X3KGG6-9/2/66a4226176a1d020d9e82b798aa85470

Biosynthesis of carbohydrate structures is tissue-specific and developmentally regulated by glycosyltransferases like fucosyl-, sialyl- and N-acetylglucosaminyltransferases. During carcinogenesis, aberrant glycosylation leads to the development of tumor subpopulations with different adhesion properties. The aim of this contribution was to directly compare mRNA expression of several glycosyltransferases in surgical specimens of gastric carcinomas. Carcinoma specimens were classified and characterized according to the WHO/UICC system. In each case, the expression of 12 glycosyltransferase enzymes was studied simultaneously by RT-PCR. For semi-quantitative analysis, amplification of the sample sequence was compared with that of [beta]-actin, co-amplified within the same tube. Expression of N-acetylglucosaminyltransferase V in gastric carcinomas was significantly enhanced compared to
normal tissue. Also, expression of sialyltransferase ST3Gal-IV and fucosyltransferase FT-IV was significantly enhanced in carcinoma tissue. No significant differences in glycosyltransferase expression were found in samples positive for Helicobacter pylori or between the different gastric regions. Thus, carcinogenesis is characterized by specific alterations in mRNA expression of several glycosyltransferases. Future studies will show whether RT-PCR detection of the expression of these enzymes could be helpful for prognostic purposes.


http://www.sciencedirect.com/science/article/B6T1W-3SXDS-7/2/26c072a5a940ffbdff35a30ca1d7378

Solution hybridization is an essential step in sequencing and some point mutation detection methods. In practice, this hybridization is hampered resulting in the need of additional purification of the amplification products. The use of T7 gene 6 exonuclease may lead to efficient production of single-stranded DNA. In this study, the effect of pretreatment with exonuclease on direct cycle sequencing and point mutation detection was analyzed. Exonuclease-treated products were directly cycle sequenced without further purification. This resulted in highly efficient quality improvement for sequencing allowing detection of heterozygotes. Point mutation detection by Point-EXACCT (exonuclease-amplification coupled capture technique) demonstrated detection of one cell containing a mutation in an excess of 75000 wild type cells. Exonuclease-enhanced detection methods offer simple, rapid detection strategies that are easily adaptable for widespread clinical laboratory use. With the use of exonuclease, the detection of heterozygosity using fluorescent cycle sequencing is becoming more reliable. The high sensitivity of Point-EXACCT due to the use of exonuclease makes it a highly promising method for large-scale screening of (pre)malignant changes in patients with a high risk for developing cancer.


http://www.sciencedirect.com/science/article/B6T1W-497C7TR-2P/2/2db500c607ac2955714dd75a0752143b

We have previously demonstrated that intake of fat as well as carbohydrate affects the activity and immunoreactive amount of sucrase-isomaltase (S-I) in rat jejunum. To examine whether diet-related changes in sucrase and isomaltase activities are accompanied by the variations of sucrase-isomaltase mRNA levels, 7-week-old rats were fed either a high-long-chain triacylglycerols diet (73 energy% as corn oil), a high-medium-chain triacylglycerols (MCT) diet (66 energy% as MCT, 7 energy% as corn oil) or a high-carbohydrate diet (70 energy% as corn starch) for 7 days. Northern blot analysis revealed that S-I mRNA levels were abundant in the jejunum of rats fed the high-MCT diet; the levels were similar to those in the rats fed the high-carbohydrate diet. Force-feeding a high-sucrose diet (40 energy% as sucrose) brought about a parallel rise in both S-I mRNA and sodium-glucose cotransporter (SGLT1) mRNA levels within 12 h. Force-feeding the high-MCT diet also produced an elevation of S-I mRNA and SGLT1 mRNA. However, force-feeding a diet containing [alpha]-methylglucoside, a non-metabolizable but actively transported sugar, did not increase S-I mRNA or SGLT1 mRNA level; sucrase activity was nevertheless elevated by feeding [alpha]-methylglucoside diet. These results suggest that not only carbohydrate intake but also MCT intake might influence S-I mRNA and SGLT1 mRNA levels in the jejunum, presumably through common metabolite(s) of carbohydrates and MCT, and that carbohydrate may play another role in enhancement of the sucrase activity through
modulation of translation and/or posttranslational modifications of the sucrase-isomaltase complex.

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http://www.sciencedirect.com/science/article/B6T1X-3PH8VKP-9/2/1a1225c75d1c6fcc94fc65b2eda637ae

Mammalian pancreatic phospholipases A2 (PLA2) have recently been implicated in cell surface receptor-mediated inflammation. As a first step toward understanding how human pancreatic PLA2 (hp-PLA2) interacts with membranes and other biological targets including cell-surface receptors, we constructed its bacterial expression vector which can be used for the mutagenesis and protein over-expression. The expression vector (pSH-hp) was constructed using a synthetic hp-PLA2 gene whose transcription is controlled by T7 promoter. hp-PLA2 was expressed as a mature protein in high concentration in Escherichia coli cells and formed inclusion body. The solubilization of inclusion body protein followed by the refolding and purification produced ca. 5 mg of pure protein from one liter of growth medium. Kinetic studies of recombinant human, bovine and porcine pancreatic PLA2s using polymerized mixed liposomes and micelles as substrates showed that despite their highly homologous structures these mammalian pancreatic PLA2s have distinct phospholipid head group specificity and different activity toward various lipid substrates.


http://www.sciencedirect.com/science/article/B6T1X-3V75GK1-8/2/82ff7406d028a5e08e2cb7405b79a76c

While UDP-glucuronosyltransferases (UGTs) are known to be expressed at high levels in human liver, relatively little is known about extrahepatic expression. In the present study, UGT2B family isoforms involved in the glucuronidation of steroid hormones and bile acids have been characterized in microsomes prepared from jejunum, ileum and colon from six human subjects. Glucuronidation of androsterone and testosterone was highly significant and increased from proximal to distal intestine. In contrast, hyodeoxycholic acid was glucuronidated at a low level in jejunum and ileum and activity was barely detectable in colon. No significant glucuronidation of lithocholic acid was found. Small phenols were glucuronidated with much lower activity than found in liver. High levels of UGT protein were detected with polyclonal anti-rat androsterone- and testosterone-UGT antibodies, whereas UGT2B4, a major hepatic hyodeoxycholic acid-specific UGT, was undetectable using a highly specific anti-human UGT2B4 antibody. Screening for RNA expression by RT-PCR confirmed the absence of UGT2B4 and UGT1A6 and showed expression of UGT2B7, a hepatic isoform shown to glucuronidate androsterone, in all intestinal segments. To our knowledge, the presence of functional androsterone and testosterone directed isoforms in human intestine is a novel finding which supports the idea that the intestinal tract functions as a steroid-metabolizing organ and plays a significant role in steroid hormone biotransformation.

http://www.sciencedirect.com/science/article/B6T1X-3S0DFXN-7/2/242b72107cc2a9f1a2b0a893d27cde2a

A cDNA fragment which encodes salmon peroxisome proliferator activated receptor [gamma] (sPPAR[gamma]) was amplified by PCR from the liver of Atlantic salmon (Salmo salar L.). The fragment was 627 bp long. The sequence of the amplified PCR product was similar to the PPAR[gamma] of mouse and hamster. 59% of the bases were identical. Northern blot analysis of salmon liver mRNA showed that the amplified sPPAR[gamma] fragment hybridised to three specific transcripts of lengths 1.6, 2.4 and 3.3 kb. Clofibrate acid and bezafibrate, administered to salmon hepatocytes in culture, resulted in a 1.7-fold increase of the 1.6 kb sPPAR[gamma] transcript. The activity of acyl-CoA oxidase also increased approx. 1.7-fold after administration of fibrates. These results indicate that PPAR is an important factor in mediating enzymatic response to fibrates in fish.


http://www.sciencedirect.com/science/article/B6T1X-497C7XB-9/2/1389ac06fc1669f3ce935ee15328ae9a

The scavenger receptors type I and II are mediators for the binding and uptake of chemically modified lipoproteins and are restricted to cells of monocyte origin. These receptors are highly expressed during the process of monocyte to macrophage differentiation. Quantitative mRNA levels of scavenger receptors from peripheral blood mononuclear cells have been analyzed in 29 hyperlipidemic patients and 15 healthy controls. Macrophage scavenger receptor isoforms transcripts were studied in circulating peripheral blood mononuclear cells with a modified RT-PCR method based on the use of a non-modified internal standard and a mathematical logistic adjustment of the standard curve. This method makes it feasible to study the variation in the expression of the scavenger receptors gene in peripheral blood during different physiopathological conditions. We studied the expression of the scavenger receptors gene in different blood cell lines and was present in only those of monocytic origin. The results have shown evidence that levels of scavenger receptor type I transcripts were proportional to apoB/cholesterol levels whereas type II receptors did not show any transcriptional variability. These findings suggest that the cholesterol level exerts a selective up-regulation of the scavenger receptor type I which is detectable by the induced increment of circulating monocytes in the blood of hyperlipidemic patients.
The genome of the nematode Caenorhabditis elegans contains several genes that appear to encode proteins similar to CTP:phosphocholine cytidylyltransferase (CCT). We have isolated a 1044-nucleotide cDNA clone from a C. elegans cDNA library that encodes the 347-amino acid version of CCT that is most similar to previously-identified CCTs. Native and His-tagged forms were expressed and purified using a baculovirus expression system. The enzyme was maximally activated by 5 [mu]M phosphatidylcholine:oleate (50:50) vesicles with a kcat value in the presence of lipid 37-fold greater than the kcat value in the absence of lipid. To localize the region of C. elegans CCT critical for lipid activation, a series of C-terminal truncation mutants was analyzed. CCT truncated after amino acids 225 or 245 was quite active in the absence of lipids and not further activated in the presence of lipids, supporting the concept that the lipid-activation segment is inhibitory to catalysis in the absence of lipids. CCT truncated after amino acids 266, 281, or 319 was activated by lipid similar to wild-type enzyme. Kinetic analysis in the absence of lipid revealed the lipid-independent CCT truncated after amino acid 245 to have a kcat value 15-fold greater than either full-length CCT or CCT truncated after amino acid 266. We conclude that elements critical for activation of C. elegans CCT by lipids are contained within amino acids 246-266, that this region is inhibitory in the absence of lipids, and that the inhibition is relieved by the association of the enzyme with lipid.
Both a 25-hydroxylation and a 1\([\alpha]\)-hydroxylation are necessary for the conversion of vitamin D3 into the calcium-regulating hormone 1\([\alpha]\),25-dihydroxyvitamin D3. According to current knowledge, the hepatic mitochondrial cytochrome P450 (CYP) 27A and microsomal CYP2D25 are able to catalyze the former bioactivation step. Substantial 25-hydroxylase activity has also been demonstrated in kidney. This paper describes the molecular cloning and characterization of a microsomal vitamin D3 25- and 1\([\alpha]\)-hydroxylase in kidney. The enzyme purified from pig kidney and the recombinant enzyme expressed in COS cells catalyzed 25-hydroxylation of vitamin D3 and 1\([\alpha]\)-hydroxyvitamin D3 and, in addition, 1\([\alpha]\)-hydroxylation of 25-hydroxyvitamin D3. The cDNA encodes a protein of 500 amino acids. Both the DNA sequence and the deduced peptide sequence of the renal enzyme are homologous with those of the hepatic vitamin D3 25-hydroxylase CYP2D25. Genomic Southern blot analysis suggested the presence of a single gene for CYP2D25 in the pig. Immunohistochemistry experiments indicated that CYP2D25 is expressed almost exclusively in the cells of cortical proximal tubules. The expression of CYP2D25 in kidney, but not in liver, was much higher in the adult pig than in the newborn. These findings indicate a tissue-specific developmental regulation of CYP2D25. The results from the current and previous studies on renal vitamin D hydroxylations imply that CYP2D25 has a biological role in kidney.


Acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes cholesterol esterification in mammalian cells. Two isoforms of ACAT have been reported to date (ACAT-1 and ACAT-2). ACAT-1 is ubiquitously expressed in tissues except the intestine. In contrast, ACAT-2 is expressed mainly in the intestine in humans. To investigate the relationship between ACAT-2 and dyslipidemia, we determined the structure of the human ACAT-2 gene and then studied the relationship between mutations of the ACAT-2 gene and dyslipidemia. To isolate human ACAT-2 genomic DNA, we designed primers based on the human ACAT-2 cDNA sequence: forward primer 5'-ACACCTCGATCTTGGTCCTGCCATA-3' and reverse primer 5'-GGAATGCAGACAGGGAGTCCT-3'. Using these primers, a human P1-derived artificial chromosome (PAC) library was screened by PCR-based procedures. Isolated PAC clones were completely digested with BamHI and subcloned into plasmid vector. Subclones that contained exons were screened by dot-blot hybridization using partial ACAT-2 cDNA fragments. The coding region of the ACAT-2 gene was encoded in 15 exons from 51 to 265 base pairs on a 21 kilobase span of genomic DNA. The exonic sequences coincided completely with that of ACAT-2 cDNA, and each exon-intron junction conserved splicing consensus sequences. Next, 187 (91 dyslipidemic and 96 normolipidemic) subjects were screened by PCR single-strand conformational polymorphism analysis of the ACAT-2 gene. Three mutations were identified by DNA sequencing: two missense mutations (E14G in exon 1 and T254I in exon 7) and a point mutation in intron 7 (-35G->A). Mutations in exon 1 and intron 7 were not associated with plasma concentrations of lipids and apolipoproteins (apo). However, plasma apoC-III levels in T254I heterozygotes were significantly higher than those in subjects without mutation. Plasma triglyceride (TG) levels in T254I heterozygotes were similar to those in subjects without mutation. Although further studies are needed, our data suggest that ACAT-2 may contribute to apoC-III gene expression and the assembly of apoC-III and TG, possibly in the intestine.

http://www.sciencedirect.com/science/article/B6VNN-42MN74WB/2/34bbbe8ce713541919d4d2f1d5be0d1e

cDNAs encoding major plasma apolipoproteins (apo) were cloned from the eel Anguilla japonica liver and their nucleotide sequences determined. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that eel lipoproteins contain apolipoproteins of 28 kDa and 14 kDa as major components. Each of the two apolipoproteins showed two isoforms having different isoelectric points as demonstrated by two-dimensional electrophoresis. The two 28 kDa components had different N-terminal amino acid sequences, whereas the two 14 kDa components had an identical one. Then cDNA clones encoding these apolipoproteins were isolated from a cDNA library constructed from the eel liver. An acidic 28 kDa component (28 kDa-1) consisted of 259 amino acids including a putative signal peptide of 27 residues, whereas a basic 28 kDa component (28 kDa-2) was composed of 260 amino acids containing a putative signal peptide of 23 residues. The tandem repeating units, which are characteristic of apolipoproteins, for 28 kDa-1 showed 27.8% identity to that of porcine apoA-IV, although mammalian apoA-IV is about 40 kDa and much larger than 28 kDa-1. However, the repeating units of 28 kDa-2 showed 52.5% identity to that of Atlantic salmon apoA-I. The 14 kDa apolipoprotein consisted of 142 amino acids containing a putative signal peptide of 20 residues. It has a novel sequence differing from apolipoproteins of other vertebrates. The transcriptional expressions of 28 kDa-1, 28 kDa-2, and 14 kDa components were all restricted to the liver, except for the transcripts of 28 kDa-2 which were also slightly expressed in the intestine.


http://www.sciencedirect.com/science/article/B6VNN-3VXS8YX-3/2/258814b7168aabf9c1739135aaf2f067

Bovine corneal epithelium contains arachidonate 12- and 15-lipoxygenase activity, while human corneal epithelium contains only 15-lipoxygenase activity. Our purpose was to identify the corneal 12- and 15-lipoxygenase isozymes. We used cDNA cloning to isolate the amino acid coding nucleotide sequences of two bovine lipoxgenases. The translated sequence of one lipoxgenase was 82% identical with human 15-lipoxygenase type 2 and 75% identical with mouse 8-lipoxygenase, whereas the other translated nucleotide sequence was 87% identical with human 12-lipoxygenase of the platelet type. Expression of 15-lipoxygenase type 2 and platelet type 12-lipoxygenase mRNAs were detected by Northern analysis. In addition to these two lipoxgenases, 12-lipoxygenase of leukocyte (tracheal) type was detected by polymerase chain reaction (PCR), sequencing, and Northern analysis. Finally, PCR and sequencing suggested that human corneal epithelium contains 15-lipoxygenase types 1 and 2.

A shift from sialylation to fucosylation of mucosal glycoconjugates occurred in the mammalian digestive tract in the weaning period, but mice under germ-free conditions were found to express both fucosyl GM1 (FGM1) and fucosyl asialo GM1 (FGA1) in the stomach, cecum and colon, but not in the small intestine. By host-microbe interactions and administration of cycloheximide, FGA1 was quickly induced in the small intestine, but the concentrations of fucosylated glycolipids in the other regions were not altered significantly. Their expression coincided with the activity of GDP-fucose:GA1 [alpha]1,2-fucosyltransferase ([alpha]1,2-FT), and we isolated a cDNA with an open reading frame encoding the murine [alpha]1,2-FT (MFUT-II) of 347 amino acids with a predicted molecular mass of 39.21 kDa. The intraperitoneal injection of cycloheximide induced the mRNA and activity of [alpha]1,2-FT (MFUT-II) in the small intestine of germ-free mice, whereas no change in the mRNA or activity was observed in the stomach, cecum and colon, indicating that expression of FGA1 in response to microbial colonization or cycloheximide is transcriptionally regulated in a restricted region of the murine digestive tract. At 24 h after the administration of cycloheximide, FGA1 was preferentially produced in the upper half of the duodenal microvilli.


The rat hepatoma-human fibroblast hybrid cell line WIF-B9 stably exhibits the structural and functional characteristics of normal differentiated hepatocytes. The abilities of these cells to synthesize bile acids and amidate them with glycine and taurine were investigated. The release of bile acids into the culture media over 72 h was assessed by gas chromatography-mass spectrometry. WIF-B9 cells were able to synthesize bile acids (1.10+/-.17 nmol/mg protein) but less efficiently than rat hepatocytes in primary culture (2.19+/-.019 nmol/mg protein; P<0.01). The patterns of major bile acid species produced by both types of cells were also different. Cholic acid (CA; 72%) and [beta]-muricholic acid (19%) were the major bile acids produced by rat hepatocytes, while chenodeoxycholic acid (CDCA) accounted for only 4.5% of total bile acids. In contrast, muricholic acids were absent, while CA (62%) and CDCA (34%) were the most abundant bile acids synthesized by WIF-B9 cells. Using reverse transcription-polymerase chain reaction and gene- and species-specific primers for key enzymes involved in bile acid synthesis, the expression of human, but not rat, orthologues of CYP7A1, CYP27, CYP8B and CYP7B1 was found in WIF-B9 cells. Induction of cell stress by serum deprivation did not change the amount of total bile acids synthesized by these cells, but an inversion of the CA-to-CDCA ratio from 1.8 to 0.3 together with a marked increase in the proportion of intermediate metabolites related to the acidic pathway was found. Using 500 [mu]M radiolabeled CA and 2 mM of taurine or glycine, the ability to amidate CA over 48 h was determined by high performance liquid chromatography. Rat hepatocytes conjugated more than 90% CA with either amino acid, whereas this ability was very poor (<2%) in WIF-B9 cells. Regarding the expression of enzymes and the products of bile acid synthesis, it may be concluded that the human phenotype predominates over that of the rat in WIF-B9 cells. Moreover, these cells are almost completely unable to further conjugate primary bile acids, which facilitates the manipulation of these steroids in analytical procedures. These characteristics make WIF-B9 cells a suitable in vitro model to carry out studies on bile acid synthesis by 'human-like' metabolic pathways.

Background: Acyl-CoA:cholesterol acyltransferase (ACAT) plays important roles in cellular cholesterol homeostasis. Two isoforms of ACAT have been reported (ACAT-1 and ACAT-2). ACAT inhibitors cannot only prevent atherosclerosis formation, but may also induce its regression in animals. In humans, an ACAT inhibitor was shown to have a lipid-lowering effect. The present study was carried out to clarify the relationship between ACAT-1 gene variants and hyperlipidemia. Methods and results: To identify genetic variants, we screened 30 subjects with hyperlipidemia by direct sequencing. As a result, a missense variant (R526G) and a variant in the 5’ untranslated region (-77G->A) were identified. The genotype frequencies of each variant were determined in 178 unrelated normolipidemic and 441 unrelated hyperlipidemic subjects. The allele frequencies of the R526G variant in normolipidemic and hyperlipidemic subjects were 0.676 and 0.633, respectively. The allele frequencies of the -77G->A variant in normolipidemic and hyperlipidemic subjects were 0.503 and 0.515, respectively. Differences in allele frequencies between normolipidemic and hyperlipidemic subjects were not significant in both variants. R526G variant did not affect plasma concentrations of lipids or apolipoproteins in subjects studied. However, among hyperlipidemic subjects, plasma concentrations of HDL-C and apoA-I in subjects with -77G->A variant were significantly higher than those in subjects without variant. Conclusion: Two variants in ACAT-1 gene were identified in subjects with hyperlipidemia. -77G->A variant affects plasma HDL concentrations only in hyperlipidemic subjects. These data suggest that the intracellular FC concentration might modulate plasma HDL concentrations.


http://www.sciencedirect.com/science/article/B6VNN-4BP3D26-1/2/a740bc0bbef84f3b740aaf1eea977257

The membrane-bound acyl-CoA elongase complex is a key enzyme responsible for erucoyl-CoA synthesis. Among the four putative genes encoding the four moieties of this complex in Brassica napus seeds, only one has been characterized, the Bn-fae1 gene, which encodes the 3-ketoacyl-CoA synthase. The genes encoding the other enzymes (3-ketoacyl-CoA reductase, 3-hydroxyacyl-CoA dehydratase and trans-2,3-enoyl-CoA reductase) have not been identified. We cloned two 3-ketoacyl-CoA reductase cDNA isoforms, Bn-krc1 and Bn-kcr2, from B. napus seeds. Their function was identified by heterologous complementation in yeast by restoring elongase activities. The comparison of Bn-kcr mRNA expression in different B. napus tissues showed that the genes were preferentially expressed in seeds and roots. We also investigated the regulation of gene expression in High Erucic Acid Rapeseed (HEAR) and in Low Erucic Acid Rapeseed (LEAR) cultivars during seed development. The co-expression of Bn-fae1 and Bn-kcr observed in HEAR cultivar during seed development was different in LEAR cultivar, suggesting that expression of both genes was directly or indirectly linked.

The Bn-FAE1.1 and Bn-FAE1.2 genes encode the 3-ketoacyl-CoA synthase, a component of the elongation complex responsible for the synthesis of very long chain monounsaturated fatty acids (VLCMFA) in the seeds of Brassica napus. Bn-FAE1 gene expression was studied during seed development using two different cultivars: Gaspard, a high erucic acid rapeseed (HEAR), and ISLR4, a low erucic acid rapeseed (LEAR). The mRNA developmental profiles were similar for the two cultivars, the maximal expression levels being measured at 8 weeks after pollination (WAP) in HEAR and at 9 WAP in LEAR. Differential expression of Bn-FAE1.1 and Bn-FAE1.2 genes was also studied. In each cultivar the same expression profile was observed for both genes, but Bn-FAE1.2 was expressed at a lower level than Bn-FAE1.1. Secondly, VLCMFA synthesis was measured using particulate fractions prepared from maturing seeds harvested weekly after pollination. The oleoyl-CoA and ATP-dependent elongase activities increased from the 4th WAP in HEAR and reached the maximal level at 8 WAP, whereas both activities were absent in LEAR. In contrast, the 3-hydroxy dehydratase, a subunit of the elongase complex, had a similar activity in both cultivars and reached a maximum from 7 to 9 WAP. Finally, antibodies against the 3-ketoacyl-CoA synthase revealed a protein of 57 kDa present only in HEAR. Our results show: (i) that both genes are transcribed in HEAR and LEAR cultivars; (ii) that they are coordinately regulated; (iii) that Bn-FAE1.1 is quantitatively the major isoform expressed in seeds; (iv) that the Bn-FAE1 gene encodes a protein of 57 kDa responsible for the 3-ketoacyl-CoA synthase activity.


The expression of acetyl-CoA carboxylase (ACC) in mouse peripheral nervous system (PNS) was investigated. Both ACC 265 and ACC 280 isoforms were expressed in the sciatic nerve, although ACC 265 was predominant. ACC 265 transcripts originating from promoters P1 and P2 could be detected in the developing nerve, as well as the two splice products, which are characterized by the presence or the absence of a 24-base sequence before the codon serine-1200. The mRNA levels for ACC 265 parallel those of other lipogenic genes whose expression is linked to the myelination process. In addition, ACC 265 mRNA and protein levels in the nerves of the trembler mutant, which is a mouse model of PNS dysmyelination, represented around 30% of the normal values. The expression of the sterol regulatory element-binding proteins (SREBPs) was also studied. SREBP 1 mRNAs were expressed at a constant level during nerve development, and their quantities were normal in trembler. On the contrary, SREBP 2 mRNA quantities varied during the myelination period similarly to the lipogenic gene mRNAs, and the levels measured in trembler represented only 10% of the normal values. Taken together, these results suggest that the coordinate expression of several lipogenic genes, which occurs during PNS myelination, could possibly be regulated by SREBP 2.
Wiskott-Aldrich syndrome (WAS) is one of the primary immunodeficiency diseases, that is inherited as an X-linked recessive trait. Since the responsible gene, the WASP gene, has been identified, various mutations for patients with WAS have been reported. We found a novel splice-site mutation in a patient with clinically diagnosed WAS. The mutation was a replacement of ag by aa in an acceptor site of intron 2 of the WASP gene. Sequencing studies of the WASP cDNA of the patient revealed that exon 3 of the WASP gene was abnormally missing due to a splicing defect.

The development of the polymerase chain reaction (PCR), which routinely can amplify specific target sequences more than one billion-fold, has made it possible to produce readily detectable amounts of DNA from a few copies of very rare sequences. We have begun a study of mitochondrial myopathies with the purpose of developing a diagnostic test using PCR to amplify appropriate mitochondrial DNA (mtDNA) target sequences from small amounts of sample. We have developed a 15-min procedure for recovering mtDNA which can be amplified by PCR to detectable levels, from as little as 30 [mu]l of blood or 5 [mu]l of amniotic fluid. We have microscopically selected HL60 cells, and have found that 28 cycles of PCR allows the detection of mitochondrial targets from a single cell. Using micromanipulation techniques, we utilized this approach to analyze mtDNA from a single cell isolated from an 8-cell stage mouse blastocyst. Finally, a single cell cultured from a patient with Leber's hereditary optic neuropathy, a mitochondrial myopathy, provided sufficient mtDNA for detection of the single base substitution that leads to loss of a restriction endonuclease recognition site for SfaNI and generation of a site for MaeIII.

In this study, we have evaluated the role of cytokine-induced neutrophil chemoattractant (CINC), in the upregulation of neutrophil Ca2+ signaling in neutrophils from thermally injured rats treated with anti-CINC antibody. Additionally, we have determined the effect of the treatment with CINC antibody on the accumulation of activated neutrophils in the intestinal wall, and the effect of such accumulation on gut bacterial translocation. Measurements of myeloperoxidase (MPO) activity and immunohistochemical localization of neutrophils determined neutrophil sequestration in the rat intestine. Agar culture analyses and a specific Escherichia coli [beta]-galactosidase gene
polymerase chain reaction was carried out to detect gut indigenous bacterial invasion into intestinal wall and extraintestinal mesenteric lymph nodes (MLN). The results showed that pretreatment of rats with anti-CINC antibody attenuated the thermal injury-induced enhancement in [Ca2+]i responses in neutrophils both in the basal and Formyl-Met-Leu-Phe stimulated conditions. Moreover, treatment with the CINC antibody decreased neutrophil infiltration into the gut and attenuated thermal injury-caused translocation of bacteria into the MLN.


Mucopolysaccharidosis BID results from the deficiency of N-acetylglucosamine 6-sulfatase activity. A Nubian goat with this lysosomal storage disease has been identified. As a first step in developing this animal model for testing treatment methods, we cloned and sequenced the caprine N-acetylglucosamine 6-sulfatase cDNA coding region. Overall there is 88% nucleotide homology between the goat and human sequence and 94% homology of the deduced amino acid sequence. The human and two ruminant species differ by the presence of an imperfect trinucleotide (CCG) repeat in the ruminant signal sequence.


Albumin Ortonovo is a slow moving variant of human serum albumin which has been found only in people coming from the small villages of Ortonovo and Nicola (Liguria, Italy) and reaches polymorphic frequency (>=1%) in the poorly admixed population group living in that area. This is the first report of a 'private' varint detected in a Caucasian population. It probably originated as a mutation in a founder individual many generations ago. Isoelectric focusing analysis of CNBr fragments from the purified variant localized the mutation in fragment CNBr (residues 447-548). This fragment was isolated on a preparative scale by reversed-phase HPLC and subjected to V8 proteinase digestion. Sequence analysis of the abnormal V8 peptide revealed that the variant arises from a previously unreported substitution at position 505 where glutamic acid has been replaced by lysine. The protein data were confirmed by DNA sequence analysis which indicated a single nucleotide change of in the corresponding codon of the structural gene. Since the amino acid substitution found in albumin Ortonovo accords with its electrophoretic mobility on cellulose acetate, residue 505 is probably exposed to the solvent. The clustering of the mutations in the intersubdomain connection linking subdomains IIIA and IIIB (residues 492-511) accords with the fact that this region lies on the molecular surface and is accessible to solvent.

LA Wistar rats have a deficiency of androsterone UDP-glucuronosyltransferase (UDPGT) and are present in Wistar rat colonies around the world. In order to clarify the molecular mechanism of the deficiency, androsterone UDPGT cDNA clone, pGT2 was isolated from rat liver cDNA library and was digested with restriction enzymes to afford three probes for Northern and Southern blot analyses in HA (normal), heterozygous LA and LA Wistar rats. In Northern blot analysis, androsterone UDPGT mRNA was totally absent in LA Wistar rat liver. Southern blot analysis suggested a large deletion of androsterone UDPGT gene in the rats. Genomic DNA amplifications with synthetic primers which have nucleotide sequences corresponding to the 5'-region of androsterone UDPGT cDNA, suggested that androsterone UDPGT gene has exon 1 with a length of some 700 bp and that this exon is deleted in LA Wistar rats. Based on these lines of evidence, it is concluded that the large portion of androsterone UDPGT gene is deleted in LA Wistar rats, which results in the absence of androsterone UDPGT mRNA and consequently the corresponding enzyme protein.


Mutations in the ATP-binding cassette transporter 1 (ABCA1) gene have been recently identified as the molecular defect in Tangier disease (TD) and familial high density lipoprotein deficiency (FHA). We here report novel mutations in the ABCA1 gene in two sisters from a Japanese family with TD who have been described previously (S. Ohtaki, H. Nakagawa, N. Kida, H. Nakamura, K. Tsuda, S. Yokoyama, T. Yamamura, S. Tajima, A. Yamamoto, Atherosclerosis 49 (1983)) and a family with FHA. Both probands of TD and FHA developed coronary heart disease. Sequence analysis of the ABCA1 gene from the patients with TD revealed a homozygous G to A transition at nucleotide 3805 of the cDNA resulting in the substitution of Asp 1229 with Asn in exon 27, and a C to T at nucleotide 6181 resulting in the substitution of Arg 2021 with Trp in exon 47. Sequence analysis of the ABCA1 gene from the FHA patient revealed a homozygous 4 bp CGCC deletion from nucleotide 3787 to 3790 resulting in premature termination by frameshift at codon 1224. These mutations were confirmed by restriction digestion analysis, and were not found in 141 control subjects. Our findings indicate that mutations in the ABCA1 gene are associated with TD as well as FHA.


Hypoxanthine phosphoribosyltransferases (HPRTs) are of biomedical interest because defects in the enzyme from humans can result in gouty arthritis or Lesch-Nyhan syndrome, and in parasites these enzymes are potential targets for antiparasite chemotherapy. In HPRTs, a long flexible loop (active site loop II) closes over the active site during the enzyme catalyzed reaction. Functional roles for this loop have been proposed but have yet to be substantiated. For the present study,
seven amino acids were deleted from loop II of the HPRT from Trypanosoma cruzi to probe the functional role of this active site loop in catalysis. The mutant enzyme ([Delta]loop II) was expressed in bacteria, purified by affinity chromatography, and kinetic constants were determined for substrates of both forward (purine salvage) and reverse (pyrophosphorolysis) reactions catalyzed by the enzyme. Loop II deletion resulted in moderate (0.6-2.7-fold) changes in the Michaelis constants (Kms) for substrates other than pyrophosphate (PPI), for which there was a 5.8-fold increase. In contrast, kcat values were severely affected by loop deletion, with rates that were 240-840-fold below those for the wild-type enzyme. Together with previously reported structural data, these results are consistent with active site loop II participating in transition-state stabilization by precise positioning of the substrates for in line nucleophilic attack and in the liberation of PPI as a product of the salvage reaction.


http://www.sciencedirect.com/science/article/B6T1Y-43YXD6-1/2/9ed986ac8633eefa0f39cac035307cfd

Caudal type homeobox gene-1 and -2 (CDX-1 and CDX-2), homologues of the Drosophila homeobox gene caudal, encode transcription factors in endoderm derived tissues of the intestine. CDX genes control proliferation and differentiation of intestinal mucosal cells and colon cancer cells. Hirschsprung's Disease (HD) or congenital intestinal aganglionosis, a major developmental anomaly of intestine, which causes functional intestinal obstruction, is frequently associated with enterocolitis. Aetiology of HD-associated enterocolitis (HDEC) remains obscure. Reduction of gut mucosal enteroendocrine cells, and inefficient transfer of the secretory immunoglobulin A across the gut mucosal cell were shown to be associated with enterocolitis in HD patients suggesting that mucosa may directly involve in the pathophysiology of HDEC. This study aims to ascertain whether the CDX-1 and CDX-2 genes, that control the proliferation and differentiation of mucosal cells, play a role in HDEC. Using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridisation, we analysed the expression of CDX-1 and CDX-2 genes in colon specimens of normal controls, necrotising enterocolitis (NEC) infants, and HD patients with and without enterocolitis. We showed for the first time that CDX-1 and CDX-2 genes were expressed in the colonic mucosal epithelium in normal, NEC and in HD infants. However, the expressions of both genes were reduced in patients with HDEC. Our findings suggest that reduced expression of CDX-1 and CDX-2 genes in mucosa may be associated with the development of HDEC.


http://www.sciencedirect.com/science/article/B6T1Y-47NVXKK-40/2/ed1c8a5d86a42e91af8b47e2bc44583d

Lysosomal [beta]-hexosaminidase (EC 3.2.1.52) occurs as two major isozymes hexosaminidase A ([alpha][beta]) and B ([beta][beta]). The [alpha] subunit is encoded by the HEXA gene and the [beta] subunit by HEXB gene. Defects in the [alpha] or [beta] subunits lead to Tay-Sachs or Sandhoff disease, respectively. While many HEXA gene mutations have been reported only three HEXB gene mutations are known. We report the characterization of two rare HEXB mutations present in genomic DNA from a single fibroblast cell line, GM203, taken from a patient with the infantile form of Sandhoff disease. The first is a single base pair deletion in exon 7 changing the
codon for Gly-258, GGA, to GA and the second, a two base pair deletion in exon 11 changes the
codons for Arg-435/Val-436, AGA/GTC, to AGTC. Each mutation produces a frame shift in the
affected allele that results in a premature stop codon 17 or 20 codons downstream, respectively.
These mutations also result in the inability to detect [beta]-mRNA by Northern blot analysis of
total mRNA. These data are consistent with the idea that the severe infantile form of Tay-Sachs
or Sandhoff disease is associated with a total lack of residual hexosaminidase A activity.

different phenotypes." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1536(2-3): 97.


In Gaucher disease patients, over 100 disease-causing mutations have been identified. For
identification of the 1504C->T (R463C) mutation it is common to use PCR-restriction
fragmentation analysis using the restriction enzyme MspI. In the present study we investigated
the reliability of this approach because accurate determination of genotypes is important in
genotype-phenotype correlations. A simple modification, i.e. using the restriction enzyme HphI
instead of MspI, revealed that type I and II Gaucher disease patients who had previously been
identified as carrying the 1504C->T mutation in fact carried the 1505G->A (IVS10-1G->A)
mutation. Sequencing of the appropriate fragment confirmed this. The PCR method easily
differentiates between these two mutations in Gaucher disease patients, thus circumventing the
need for sequencing procedures. The phenotypes of the patients found to be carrying the 1505G->A
mutation are also described.

Pecina, P., M. Capkova, et al. (2003). "Functional alteration of cytochrome c oxidase by SURF1
mutations in Leigh syndrome." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1639(1): 53.

http://www.sciencedirect.com/science/article/B6T1Y-497H5B5-2/2/edf143727bab4f946b55d8d2c8f761cb

Subacute necrotising encephalomyopathy (Leigh syndrome) due to cytochrome c oxidase (COX)
deficiency is often caused by mutations in the SURF1 gene, encoding the Surf1 protein essential
for COX assembly. We have investigated five patients with different SURF1 mutations resulting in
the absence of Surf1 protein. All of them presented with severe and generalised COX defect.
Immunoelectrophoretic analysis of cultured fibroblasts revealed 85% decrease of the normal-size
COX complexes and significant accumulation of incomplete COX assemblies of 90-120 kDa.
Spectrophotometric assay of COX activity showed a 70-90% decrease in lauryl maltoside (LM)-
solubilised fibroblasts. In contrast, oxygen consumption analysis in whole cells revealed only a
13-31% decrease of COX activity, which was completely inhibited by detergent in patient cells but
not in controls. In patient fibroblasts ADP-stimulated respiration was 50% decreased and
cytofluorometry showed a significant decrease of mitochondrial membrane potential [Delta][Psi]m
in state 4, as well as a 2.4-fold higher sensitivity of [Delta][Psi]m to uncoupler. We conclude that
the absence of the Surf1 protein leads to the formation of incomplete COX complexes, which in
situ maintain rather high electron-transport activity, while their H+-pumping is impaired. Enzyme
inactivation by the detergent in patient cells indicates instability of incomplete COX assemblies.

http://www.sciencedirect.com/science/article/B6T1Y-41JTMXY-7/2/f42305d489710cdb9bf77501c1ac8f3

We have investigated the mRNA amounts of six lysosomal proteins ([beta]-hexosaminidase [alpha]- and [beta]-subunit, sphingolipid activator protein precursor, GM2 activator protein, lysosomal sialidase, [beta]-glucocerebrosidase) involved in the degradation of glycosphingolipids. We analyzed extracts from brain tissues of mouse models for lysosomal storage diseases, i.e., the GM2 gangliosidoses and the deficiency of the sphingolipid activator protein precursor (prosaposin). The mRNA levels were quantified by real-time reverse transcription-polymerase chain reaction. Although storage of the respective lysosomal proteins has been reported in human and mice, no increase of their mRNA amounts could be detected here. Our results indicate that there is no transcriptional upregulation of lysosomal proteins in the examined neuronal storage disorders.


http://www.sciencedirect.com/science/article/B6T1Y-4BJK5R5-2/2/0470444f4ec5cc25b0c18680ab058bd7

Pro-inflammatory cytokine release after shock is central in the development of subsequent multiple organ dysfunction syndrome. Some studies suggest that interleukin-10 (IL-10) is an immunosuppressive mediator after injury or sepsis, while others suggest that IL-10 is an important regulator of the pro-inflammatory response. We hypothesized that in a model of trauma and hemorrhagic shock (TH), IL-10 regulates pro-inflammatory cytokine activity via an autocrine effect on cytokine mRNA transcription in Kupffer cells early after TH. To study this, male C3H/HeN mice were sham-operated or subjected to TH. Plasma levels of TNF-[alpha], IL-6 and PGE2 were elevated following TH. A sharp peak in IL-10 levels was observed at 2 h after the insult. Kupffer cell (KC) depletion prior to TH reduced plasma IL-6, IL-10 and TNF-[alpha] levels, whereas treatment with anti-IL-10 after TH increased IL-6 and TNF-[alpha] levels. Kupffer cell mRNA expression for IL-6, IL-10 and TNF-[alpha] was elevated in the TH group and further increased by anti-IL-10 treatment. These findings indicate that KC-dependent IL-10 regulates the early systemic inflammatory response after TH. Thus, while IL-10 is an important mediator of immunosuppression following traumatic injury, it also is beneficial with regard to its ability to counter-regulate the early inflammatory response under such conditions.


http://www.sciencedirect.com/science/article/B6T1Y-46HWMW6-1/2/ccb490947e72743655c48e57ccabc678

We applied RNA arbitrarily primed-PCR (RAP-PCR) to screen the genes differentially expressed between common congenital heart defects (CHD) [atrial septal defect, ventricular septal defect, Tetrology of Fallot (TOF)] and normal human heart samples. Three of these differentially
amplified fragments matched cDNA sequences coding for proteins of unknown function in humans: hCALO (human homologue of calossin), NP79 (coding for a nuclear protein of 79KD) and SUN2 (Sad-1 unc-84 domain protein 2). The other four fragments were from known human genes: apolipoprotein J, titin, dystrophin and protein kinase C-delta. Northern blot analysis confirmed that all of these genes are expressed in the human heart. The results of RAP-PCR were reconfirmed by quantitative RT-PCR in TOF and control heart samples. Both techniques showed the levels of expression of hCALO, NP79 and SUN2 to be comparable in TOF and control samples and the level of expression of dystrophin and titin, both coding for cytoskeletal proteins, to be significantly upregulated in TOF samples. In summary, we have shown that the RAP-PCR technique is useful in the identification of differentially expressed gene from biopsy samples of human CHD tissues. In this manner, we have identified three novel genes implicated in the normal function of the human heart and two known genes upregulated in TOF samples.


http://www.sciencedirect.com/science/article/B6T1Y-3V3HPH6-8/2/4e5ead629e0f28c8cc095210ffa3624a

Mutations in genes encoding presenilin-1 (PS1) and presenilin-2 (PS2) have been linked to familial forms of Alzheimer's disease (AD). Cells expressing mutant presenilins produce elevated levels of A[beta]42, the major amyloid peptide found in AD plaques. The mechanism whereby this occurs remains unknown, but the localization of presenilins to endoplasmic reticulum (ER) and Golgi compartments has suggested that they may function in intracellular trafficking pathways involved in processing [beta]-amyloid precursor proteins (APP). To test this possibility, we coexpressed PS1(wt), PS1(M146L), or PS1(L286V) in HEK293 cells together with the LDL receptor, a classic glycoprotein marker that undergoes post-translational O-glycosylation in the Golgi compartment. Pulse-chase analysis of the receptor indicated that mutant presenilins had no effect on ER->Golgi transport. Similar results were obtained when the studies were carried out with cells expressing the Swedish variant of APP (SWAPP751) instead of the LDL receptor. Moreover, secretion of the soluble exodomain polypeptide fragments of SWAPP751 that arise from [alpha]-secretase and [beta]-secretase cleavage was not markedly affected by the PS1 mutants. Despite the lack of discernible effect of the PS1 mutants on trafficking of proteins through the Golgi apparatus, they caused a substantial increase in the proportion of A[beta]42 relative to total A[beta] in the culture medium. The results suggest that mutant forms of PS1 cause elevated production of A[beta]42 by a mechanism that is independent of a major disruption of exocytic trafficking of APP.


http://www.sciencedirect.com/science/article/B6T1Y-4BH6H52-1/2/6740e6b32030fcfd307b5eee52d8bb7

Most gastrointestinal stromal tumors (GISTs) contain activating mutations of the proto-oncogene c-kit. The GNNK- isoform of c-kit has a greater oncogenic potential than the GNNK+ isoform. We studied tumors from 29 patients with GIST, 19 of whom had c-kit mutations, and compared them to normal cells and HMC-1 mast cell line. c-kit transcripts were quantified by real-time PCR. The ratios of GNNK-/+ isoforms and of wild-type/mutant alleles were determined by RT-PCR and fluorometric quantification. On average, GISTs contained 1.9 times more c-kit transcripts than the
HMC-1 cell line and GISTs with c-kit mutations contained 2.8 times more c-kit transcripts than those without (P=0.003). The median GNNK-/+ isoform ratios in GISTs with and without c-kit mutations were 4.4 and 4.1, respectively, and there was no difference in the GNNK-/+ ratios between the GISTs and the control samples. Both mutant and wild-type alleles of c-kit were expressed in similar amounts in 13/15 mutant GISTs. The oncogenic effects of KIT in GISTs are not related to the higher expression level of the GNNK- isoform. The high expression level of both mutated and wild-type allele transcripts of c-kit suggests that interactions between spontaneously activated and normal c-kit receptors are important in GIST tumorigenesis.


Inhalation of urban pollutants elevates the circulating levels of the vasoactive peptides endothelin (ET)-1 and ET-3 in rats. This effect could explain the association between episodic variations of urban pollutants and acute cardiopulmonary morbidity and mortality documented in epidemiological studies. Because the lungs are the primary source of circulating ET-1 and the main site of clearance from circulation, we investigated the response of endothelin system genes in the lungs of Fischer-344 rats after 4-h nose-only inhalation of 0.8 ppm ozone plus 49 mg/m3 EHC-93 (Ottawa particles). The mRNA levels for preproET-1, preproET-3, endothelin-converting enzyme (ECE)-1, and ET receptor subtypes A and B were determined at 2 h, and 1, 2, 3, 7, and 14 days after exposure. The pollutants induced preproET-1 and ECE-1 (PP<0.05), and returned to control levels by 24 h, indicating that induction of ET-3 in the lungs is not responsible for the sustained elevation of ET-3 in plasma reported after inhalation of pollutants. Our results indicate that lung endothelin system genes respond rapidly and transiently to inhalation of urban pollutants, consistent with the dynamics of urban pollutant health effects in the human population.


Elevated plasma levels of homocysteine have been shown to interfere with normal cell function in a variety of tissues and organs, such as the vascular wall and the liver. However, the molecular mechanisms behind homocysteine effects are not completely understood. In order to better characterize the cellular effects of homocysteine, we have searched for changes in gene expression induced by this amino acid. Our results show that homocysteine is able to induce the expression and synthesis of the tissue inhibitor of metalloproteinases-1 (TIMP-1) in a variety of cell types ranging from vascular smooth muscle cells to hepatocytes, HepG2 cells and hepatic stellate cells. In this latter cell type, homocysteine also stimulated [alpha]1(I) procollagen mRNA expression. TIMP-1 induction by homocysteine appears to be mediated by its thiol group. Additionally, we demonstrate that homocysteine is able to promote activating protein-1 (AP-1) binding activity, which has been shown to be critical for TIMP-1 induction. Our findings suggest that homocysteine may alter extracellular matrix homeostasis on diverse tissue backgrounds besides the vascular wall. The liver could be considered as another target for such action of homocysteine. Consequently, the elevated plasma levels of this amino acid found in different pathological or nutritional circumstances may cooperate with other agents, such as ethanol, in the
onset of liver fibrosis.


http://www.sciencedirect.com/science/article/B6T1Y-47NVXTM-5P/2/7e64b1ec564ec828e03c85dab2cb31fb

An aberrant ferrochelatase mRNA lacking exon 10 was found in a patient with erythropoietic protoporphyria (EPP). In her genomic DNA an A -> T transversion at position -3 of the donor site of intron 10 appeared to be responsible for the exon skipping. Both the patient and her sister were heterozygous for this mutation.

Wanner, R., A. Panteleyev, et al. (1996). "Retinoic acid affects the expression rate of the differentiation-related genes aryl hydrocarbon receptor, ARNT and keratin 4 in proliferative keratinocytes only." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1317(2): 105.

http://www.sciencedirect.com/science/article/B6T1Y-3W2V6F4-5/2/c3c4be7f8f2644ed41870e39708296e3

The environmental contaminant dioxin exerts most of its effects by activating the aryl hydrocarbon receptor (AhR). The AhR is considered to play not only a role in the regulation of xenobiotic metabolism, but also for development, growth, and differentiation. The transcript levels of the AhR and its associated translocator protein (ARNT) were found to increase with ongoing differentiation in the human keratinocyte cell line HaCaT. Correspondingly, in situ hybridization studies in normal human skin revealed an absence of AhR-expression in proliferating basal cells and increasing transcript levels in upper cell layers, in dependence of keratinocyte differentiation. AhR expression in differentiation-deficient hyperproliferative psoriatic skin was markedly decreased. When keratinocytes were continuously treated with 1 [mu]M retinoic acid (RA), the upregulation of AhR- and ARNT-mRNA levels was inhibited as was keratin 4-expression, a marker of HaCaT-keratinocyte differentiation. In contrast, treatment of already differentiated cells with RA did not down-regulate these transcript levels. The mRNA levels of the prevalent retinoic acid receptors in keratinocytes, RAR[gamma] and RXR[alpha], were not influenced by the process of differentiation or by addition of RA. Our data suggest that the regulation of AhR-, ARNT- and keratin 4-expression by RA is indirect and mediated by a yet to be identified factor.

Biochimica et Biophysica Acta (BBA) - Molecular Cell Research (15)


http://www.sciencedirect.com/science/article/B6T20-3X52KFM-
We produced recombinant human thrombin mutants to investigate the correlation between the thrombin enzyme and mitogenic activity. Single amino acid substitutions were introduced in the catalytic triad (H43N, D99N, S205A, S205T), in the oxy-anion binding site (G203A) and in the anion binding exosite-1 region (R73E). Proteins were produced as prethrombin-2 mutants secreted in the culture medium of DXB11-derived cell lines. All mutants were activated by ecarin to the corresponding thrombin mutants; the enzymatic activity was assayed on a chromogenic substrate and on the procoagulant substrate fibrinogen. Mutations S205A and G203A completely abolished the enzyme activity. Mutations H43N, D99N and S205T dramatically impaired the enzyme activity toward both substrates. The R73E mutation dissociated the amidolytic activity and the clotting activity of the protein. The ability of thrombin mutants to induce proliferation was investigated in NIH3T3 mouse fibroblasts and rat cortical astrocytes. The ability of the thrombin mutants to revert astrocyte stellation was also studied. The mitogenic activity and the effect on the astrocyte stellation of the thrombin mutants correlated with their enzymatic activity. Furthermore the receptor occupancy by the inactive S205A mutant prevented the thrombin effects providing strong evidence that a proteolytically activated receptor is involved in cellular responses to thrombin.

Denizot, Y., A. Besse, et al. (1999). "Interleukin-4 (IL-4), but not IL-10, regulates the synthesis of IL-6, IL-8 and leukemia inhibitory factor by human bone marrow stromal cells." *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1449**(1): 83.

http://www.sciencedirect.com/science/article/B6T20-3VTSHS8-7/2/bfa57109817811134feecbe23bedd238

Leukemia inhibitory factor (LIF), interleukin 6 (IL-6) and IL-8 are important regulators of inflammation and hematopoiesis. Human bone marrow stromal cells regulate marrow hematopoiesis by secreting cytokines. By using reverse-transcriptase polymerase chain reaction (RT-PCR), we demonstrate that human bone marrow stromal cells constitutively express LIF, IL-6 and IL-8 transcripts. By using specific ELISAs, we found that their spontaneous productions of LIF, IL-6 and IL-8 are elevated in response to serum and after stimulation with the pro-inflammatory cytokines IL-1[alpha] and TNF-[alpha]. The anti-inflammatory cytokine IL-4 reduces their serum- and cytokine-induced LIF secretion. By contrast, IL-4 stimulates their serum- and IL-1[alpha]-induced IL-6 synthesis. IL-4 has no effect on the serum-induced IL-8 synthesis by marrow stromal cells, but stimulates their cytokine-induced IL-8 production. The anti-inflammatory cytokine IL-10 has no effect on the serum- and cytokine-induced LIF, IL-6 and IL-8 synthesis by bone marrow stromal cells. RT-PCR experiments reveal the presence of IL-4 receptor [alpha]-chain mRNA and IL-10 receptor mRNA in cultured bone marrow stromal cells. The differential regulation by IL-4 of two related cytokines, such as LIF and IL-6, and the enhanced effect of this 'anti-inflammatory' cytokine on IL-6 and IL-8 synthesis highlight the tightly controlled regulation and the complexity of the cytokine production within the human bone marrow.


http://www.sciencedirect.com/science/article/B6T20-497C80KC2/1/e792cd0b941fc69b6c1262c32e67bc3

Two phosphatidylinositol 4-kinase isozymes, type 3 and type 2, have been separated on
hydroxylapatite after solubilizing bovine brain microsomes with Triton X-114. Employing a newly developed renaturation procedure following SDS-PAGE, we demonstrate that a 200 kDa polypeptide carries the enzymic activity of this type 3 isoform. Chromatography on hydroxylapatite, Heparin-Sepharose, Superdex 200 and finally SDS-PAGE results in an approximately 30000-fold purification. Tryptic peptides generated from the 200 kDa polypeptide after SDS-PAGE have been sequenced and the obtained data have been used for constructing and synthesizing degenerated oligonucleotides. Polymerase chain reaction as well as screening of cDNA libraries allowed several clones to be isolated from which a 4.7 kb contiguous sequence can be built up. The open reading frame covers 4.4 kb with a 0.3 kb untranslated 3' end which yields a deduced amino acid sequence of 1,467 amino acids. The C-terminal part of ca. 300 amino acids represents the catalytic domain. Sequence alignment of this domain with the mammalian counterpart, the human type 2 phosphatidylinositol 4-kinase, the yeast kinases STT4 and PIK1, as well as with the catalytic domains of bovine, human, mouse and yeast phosphatidylinositol 3-kinases reveals a high degree of identity: 26 of these approximately 300 amino acids are invariable in all of these eight catalytic domains. Five motifs indicate nuclear localization and DNA binding properties of the enzyme. Two leucine zippe motifs (amino acids 358-386, 862-882) are detectable. Furthermore, a helix loop helix motif (amino acids 716-729) as well as two nuclear localization signals (amino acids 838-854, 345-349) indicate the presence of the type 3 isoform in the nucleus.


http://www.sciencedirect.com/science/article/B6T20-47N5FDM-1/2/c3e3f44acf59a6289062e4352eade1ae

The YIPP (tyrosine-isoleucine-proline-proline, amino acids 319-322) motif within the C-terminal part of the human AT1 receptor is associated with angiotensin II (AII)-induced activation of the Jak-STAT pathway and phospholipase C[gamma]1 phosphorylation. We report here that mutations of the YIPP motif strongly affect ligand-binding to the receptor. We analysed AT1 receptors of the wild type (WT) and 11 mutants with a FLAG-epitope-tag within their C-terminal portion. Mutations of the "P-P" amino acid sequence of this motif decreased both AII binding and the AII-induced intracellular Ca2+ transients. Mutant and WT receptors were expressed equally in the cell membrane and were localized within the plasma membrane. These results suggest that the "P-P" amino acid sequence within the YIPP motif is important for AII binding to the AT1 receptor.


http://www.sciencedirect.com/science/article/B6T20-3W26B43-N/2/7f8b88786f0a9d73915a2e96d397cf49

Human tissue contents of gliostatin/platelet-derived endothelial cell growth factor (PD-ECGF) and its drug-induced expression in tumor cells were currently examined by a sandwich enzyme immunoassay (EIA) system and a reverse transcription-polymerase chain reaction (RT-PCR) method. Gliostatin/PD-ECGF was found to distribute in rather ubiquitous than specific human tissues and organs, with a relatively high levels in the tissues of digestive system (esophagus and rectum), brain, spleen, bladder and lung, but not in gall bladder, aorta, muscle, fat and kidney. Most of examined human tumor cell lines showed 4- or 5-fold higher contents (21.5 +/- 3.9 ng/mg
protein) than normal tissue contents (4.4 +/- 1.1 ng/mg protein) on the average. While gliostatin/PD-ECGF is known to lack a signal sequence, some tumor cells (A431 and MKN74) appeared to release it into the conditioned medium. Expression of gliostatin/PD-ECGF in epidermoid carcinoma cell (A431) and stomach cancer cell (MKN45) was induced by dibutyryl cyclic AMP and phorbol ester, and uniquely in MKN45 by hydrocortisone. In particular, this hydrocortisone specifically caused an increase of the apparent secretion of MKN74 without its cytotoxic effects, suggesting a possible secretion of gliostatin/PD-ECGF in the restricted but not universal cell line. Biological significance on the chemical induction of gliostatin/PD-ECGF in tumor cells and on its extracellular secretion are discussed.


http://www.sciencedirect.com/science/article/B6T20-3WD5BRC-8/2/2b425d1beef3b5e914a0579d4f355

In the course of our examination of proteins associated with the GLUT4-containing vesicles of rat adipocytes we have identified a new 22 kDa member of the family of endoplasmic reticulum (ER) proteins known as reticulons. The protein, which we refer to as vp20, was purified from a preparation of GLUT4-containing vesicles of rat adipocytes, and tryptic peptides were micro-sequenced. From this information a cDNA encoding a single open reading frame for a protein of 22 kDa was cloned. This protein is homologous to known members of the reticulon protein family. vp20 has two hydrophobic stretches of about 35 amino acids that could be membrane spanning domains and an ER retention motif at its carboxy-terminus. vp20 was most abundant in the high density microsome fraction of adipocytes, which is the fraction most enriched in ER. Only a small fraction of vp20 was present in the GLUT4 vesicle population, and that fraction appears to be due to ER vesicles that were non-specifically bound to the adsorbent. Analysis of tissue distribution of vp20 in rats revealed that it is concentrated in muscle, fat and the brain.


http://www.sciencedirect.com/science/article/B6T20-47S6DP2-FW/2/a9b70960ae6b1999931366df035ad89

The folate receptor (FR), an essential component in the process of folate uptake in various cells, is known to exist in three isoforms, FR-[alpha], Fr-[beta] and FR-[gamma], with differential tissue expression. Transfer of folate across the human placenta from mother to fetus involves participation of a folate receptor expressed in the syncytiotrophoblast, but the isoform identity of this receptor has not been established. Based on the tissue/cell type from which these isoforms have been cloned, it is currently believed that FR-[alpha] is the isoform expressed in adult tissues whereas FR-[beta] is the isoform expressed in fetal tissues including placenta. The present study, undertaken primarily to establish the isoform identity of the FR expressed in the placental syncytiotrophoblast, does not support this currently prevailing nomenclature. Reverse transcription coupled with polymerase chain reaction (RT-PCR) of total/poly(A)+ RNA from placenta, cultured trophoblast cells and JAR choriocarcinoma cells with primer pairs specific for either FR-[alpha] or FR-[beta] reveals that while both isoforms are detectable in the whole placental tissue, only FR-[alpha] is present in the normal trophoblast cells and in the choriocarcinoma cells. Northern analysis with probes designed to distinguish between the mRNA transcripts coding for these two isoforms corroborate the RT-PCR findings. Furthermore, the
nucleotide sequences of the PCR products obtained from the trophoblast cells and JAR cells are identical to the nucleotide sequence of the FR-[alpha] cDNA. These studies establish that it is the FR-[alpha] isoform, and not the FR-[beta] isoform, which is selectively expressed in the placental trophoblast cells. FR-[beta], which is known to be present in the placenta, most likely arises from the maternal decidua normally associated with this tissue.


http://www.sciencedirect.com/science/article/B6T20-3X52KFM-P/2/09b3a198df770046fecb2a5ab256b2de

Constitutive nitric oxide synthase (cNOS) may play an important protective role in the intestine, since our previous study has shown that the degree of bowel injury induced by platelet-activating factor (PAF), a potent inflammatory mediator, is inversely related to the cNOS content of the intestine. This study aims to examine the composition of the cNOS system in rat small intestine, and its regulation by PAF. We found that an approximately 120 kDa NOS I (neuronal NOS) is the predominant NOS in rat intestine, as evidenced by the following: (a) immunoblotting with specific antibodies detected a NOS I of approximately 120 kDa, but little NOS III; (b) the Ca2+-dependent, constitutive NOS (cNOS) activity of the rat intestine was removed by immunoprecipitation with the anti-NOS I, but not anti-NOS II or anti-NOS III antibodies; (c) RT-PCR revealed constitutive expression of NOS I in the intestinal tissue, but only a minute amount of NOS III. Immunofluorescent staining with anti-NOS I located NOS in the Auerbach plexus and nerve fibers in the muscle layer. We also found that this 120 kDa NOS I is rapidly (within 1 h) down-regulated in response to PAF administration. The protein level, enzyme activity as well as mRNA of nNOS were all decreased in the intestine.


http://www.sciencedirect.com/science/article/B6T20-4DKD4XW-1/2/3c0b54bb55aabc8c47b7bb4f33a2ef8b

Differentiation of Drosophila Schneider cells caused by DNA double-strand break (DSB)-inducing topoisomerase II (topo II) inhibitors were attenuated by ICRF-193, a non-DNA-damaging topo II inhibitor. ICRF-193 did not inhibit differentiation induced by neocarzinostatin (NCS), a drug that causes DNA DSBs independent of topo II. Schneider cells differentiated upon treatment with [gamma]-ray. These results suggest that DNA DSBs induce myogenic differentiation of Schneider cells. We also found DNA replication inhibitors, hydroxyurea (HU), aphidicolin, and ethylmethanesulfonate (EMS) induced myogenic differentiation of Schneider cells. HU-induced differentiation was inhibited upon pretreatment of cells with chemical inhibitors of PP 1/2A, p38 MAPK, JNK, and proteasome. RT-PCR analysis revealed that the expressions of fusion-competent myoblast-specific genes lmd,sns, and del were induced in Schneider cells upon treatment with NCS or HU, whereas expressions of three founder cell-specific genes, duf, ants, and rol, were undetectable. These results indicate that the expression of fusion competent-myoblast-specific genes is induced during myogenic differentiation of Drosophila Schneider cells by DNA DSBs or replication inhibition.


The mechanism(s) involved in immortalization that constitute the first step during malignant transformation has been the subject of our interest. By the use of spontaneously immortalized mouse embryonic fibroblasts we have earlier identified two stages of immortalization which are characterized by growth characteristics of the cells, their conditioned medium and the protein markers such as p53, p81 and mortalin (Kaul et al. (1994) Biochim. Biophys. Acta, in press). The present study was planned to purify the mitogenic factors from the conditioned medium of stage II cells. Sequential purification by chromatography followed by peptide sequencing has characterized one of these as vascular endothelial growth factor (VEGF). Further analysis by RT-PCR suggests that the spontaneously immortalized stage II fibroblasts have enhanced synthesis and secretion of VEGF as compared to their mortal parent cells. Expression of a novel 304 bp long form of VEGF is identified in immortal fibroblasts in addition to the three known alternatively spliced forms. The study points to the involvement of VEGF function during spontaneous immortalization of mouse embryonic fibroblasts.


http://www.sciencedirect.com/science/article/B6T20-461XR21-1/2/5ea5a1d3dd20db8a76779015d92954ae

X-chromosome inactivation is a phenomenon by which one of the two X chromosomes in somatic cells of female mammals is inactivated for life. The inactivated X chromosomes are covered with Xist (X-inactive specific transcript) RNA, and also enriched with the histone H2A variant, macroH2A1.2. The N-terminal one-third of macroH2A1.2 is homologous to core histone H2A, but the function of the C-terminal two-thirds, which contains a basic, putative leucine zipper domain, remains unknown. In this study, we tried analyzing protein-protein interaction with a yeast two-hybrid system to interact with the nonhistone region of mouse macroH2A1.2. The results showed that macroH2A1.2 interacts with mouse nuclear speckled type protein Spop. The Spop protein has a unique composition: an N-terminal MATH, and a C-terminal BTB/POZ domain. Further binding domain mapping in a glutathione-S-transferase (GST) pull-down experiment revealed that macroH2A1.2 binds the MATH domain of Spop, which in turn binds to the putative leucine zipper domain of macroH2A1.2.


http://www.sciencedirect.com/science/article/B6T20-3SY9PM9-C/2/2d6714062fcc3894641c6921237c2868

The chicken melanocortin 2-receptor (MC2-R) gene was isolated. It is found to be a single copy gene encoding a 357 amino acid protein, sharing 65.8-68.7% identity with mammalian counterparts. The chicken MC2-R mRNA is expressed in the adrenal and spleen, suggesting that
the receptor mediates both endocrine and immunoregulatory functions of ACTH in the chicken. The amino acid sequence of the chicken MC2-R is collinear with those of other subtypes of MC-R, whereas all cloned mammalian MC2-Rs contain a gap in the third intracellular loop, suggesting that mammalian MC2-R molecules have evolved by lacking a part of the domain which determines the specificity of signal transduction in G-protein coupled receptors. Interestingly, the codon usage differs dramatically between MC1-R and MC2-R in the chicken; the GC-contents at the third codon position in MC1-R and MC2-R are 94.6 and 50.6%, respectively. It may reflect selective constraints on the usage of synonymous codons.


http://www.sciencedirect.com/science/article/B6T20-3WWDJM2-V/2/578fa5252f1791dfa3134386cf0fe314

The gene for pro-opiomelanocortin (POMC), a common precursor of melanocortins, lipotropins and [beta]-endorphin, was isolated in the chicken first among avian species. The chicken POMC gene was found to be a single copy gene and appeared to show the same structural organization as that of other species of different classes. The predicted POMC displayed the highest identity to Xenopus POMC(A) (60.1%), and consisted of 251 amino acid residues with nine proteolytic cleavage sites, suggesting that it could be processed to give rise to all members of the melanocortin family, including adrenocorticotrophic hormone and [alpha]-, [beta]- and [gamma]-melanocyte-stimulating hormones, as well as the other POMC-derived peptides. RT-PCR analysis detected the POMC mRNA in the brain, adrenal gland, gonads, kidney, uropygial gland and adipose tissues, each of which has been demonstrated to express melanocortin receptors. These results suggest that melanocortins act in a paracrine and/or autocrine manner to control a variety of functions both in the brain and in the peripheral tissues in the chicken.


Agouti-related protein (AGRP) is a naturally occurring antagonist of melanocortin action. It is expressed mainly in the arcuate nucleus where it plays an important role in the hypothalamic control of feeding and energy homeostasis by antagonism of central melanocortin 4 receptors in mammals. Besides in the brain, the melanocortin 4 receptor is expressed in numerous peripheral tissues in the chicken. To examine whether or not the peripheral melanocortin 4 receptor signaling could be regulated by AGRP, we cloned and localized the expression of the AGRP gene in the chicken. The chicken AGRP gene was found to encode a 154 or 165 amino acid protein, depending on the usage of two alternative translation initiation sites. The coding sequence consisted of three exons, like that of mammalian species. The C-terminal cysteine-rich region of the predicted AGRP displayed high levels of identity to mammalian counterparts (78-84%) and all 10 cysteine residues conferring functional conformation of AGRP were conserved; however, other regions showed apparently no homology, suggesting that biological activities of AGRP are located in its C-terminal region. RT-PCR analysis detected the AGRP mRNA in all tissues examined: the brain, adrenal gland, heart, liver, spleen, gonads, kidney, uropygial gland, skeletal muscle and adipose tissues. Interestingly, the skin also expressed the AGRP mRNA, where Agouti, another melanocortin receptor antagonist regulating hair pigmentation, is
expressed in rodents. Most of those AGRP-expressing tissues have been demonstrated to express melanocortin 4 receptors and/or other subtypes of melanocortin receptor whose mammalian counterparts can bind AGRP. These results imply the possibility that some peripheral melanocortin systems could be regulated by the functional interaction between melanocortins and AGRP at melanocortin receptors in the chicken.


http://www.sciencedirect.com/science/article/B6T20-4698RP3-2/2/c10a38ebd47ef95eac60cf953b59a41a

Chemokine-like factor 1 (CKLF1) is a novel cytokine first cloned from U937 cells. It contains different splicing forms and has chemotactic effects on a wide spectrum of cells both in vitro and in vivo; it can also stimulate the regeneration of skeletal muscle cells in vivo, but the mechanism remains unclear. To probe the myogenesis function of CKLF2, which is the largest isoform of CKLFs, C2C12 murine myoblasts were stably transfected with human CKLF2 eukaryotic expression vector. Compared with control vector transfected C2C12 cells, CKLF2 overexpression causes accelerated myoblast proliferation as determined by cell counting and [3H]TdR incorporation assays. In addition, CKLF2 overexpression also promotes cell differentiation, which was determined by higher expression levels of myogenin, creatine kinase, myosin and the accelerated myoblast fusion. Further analysis also indicates that CKLF2 could activate the transcription activity of the bHLH/MyoD and MEF2 families. Finally, DNA synthesis and myotube formation could also be promoted by growing C2C12 cells in conditioned media from CKLF2-transfected cells. These findings strongly suggest a role for human CKLF2 in regulation of skeletal muscle myogenesis.

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http://www.sciencedirect.com/science/article/B6T21-3V5MPS1-B/2/7d1ba0d6a3b6d5b59bfaa226f5b178a

Inositol(1,3,4,5)tetrakisphosphate (InsP4) and phosphatidylinositol(3,4,5)trisphosphate (PtdInsP3) are two potential second messengers with a still largely unknown mode of action. We recently cloned the 42 kDa protein p42IP4 previously purified from pig cerebellum, which binds InsP4 (Kd ~2 nM) and PtdInsP3 with comparable affinities (Stricker et al., FEBS Lett. 405 (1997) 229). The protein p42IP4 (pig) is highly homologous to centaurin-[alpha], a larger protein of 46 kDa, derived from a rat brain cDNA library clone (Hammonds-Odie et al., J. Biol. Chem. 271 (1996) 18859). Here we investigated whether also p42IP4 is expressed in rat brain and how it might be related to centaurin-[alpha]. When we carried out RT-PCR using mRNA from brain of rats of different ages we obtained several clones corresponding to p42IP4, but not to centaurin-[alpha]. The existence
of p42IP4 in rat brain is supported by the following findings: (1) biochemical analysis of the purified rat brain protein shows inositol phosphate ligand affinities identical to those of the protein from other species; (2) Western blot analysis of rat brain membrane fractions using a peptide-specific antiserum revealed only the 42 kDa protein (p42IP4), but did not give evidence for the occurrence of a larger 46 kDa centaurin-[alpha]-like protein in rat brain; and (3) the amino acid sequences deduced from p42IP4 cDNA are highly homologous in several species and are confirmed by protein fragment microsequences. Thus, p42IP4 from rat brain which has two pleckstrin homology domains is a protein largely conserved between different species and most likely has an important function in inositol phosphate or inositol lipid signal transduction.


http://www.sciencedirect.com/science/article/B6T21-3W7XBPV-8/2/5def93440cd3f56d0e92aae8e5ceeb98

Cathepsin D was purified to homogeneity from the liver of Antarctic icefish by anion-exchange chromatography followed by affinity chromatography on concanavalin-A Sepharose. The purified enzyme showed a molecular mass of 40 kDa and displayed optimal activity at pH 3.0 with a synthetic chromogenic substrate. The N-terminal sequence of this proteinase was determined by automated Edman degradation and was used to design a primer for use in reverse-transcriptase polymerase chain reaction. The open reading frame of the cloned cDNA encoded an aspartic proteinase, which contained the experimentally determined N-terminal sequence. The predicted sequence (396 residues) had a high similarity with those of cathepsin D from various vertebrate sources, but was considerably different from that of nothepsin, a distinct aspartic proteinase described previously from Antarctic fish [1]. Determination of kinetic parameters for substrate hydrolysis showed that, at temperatures between 8 and 50[deg]C, the icefish cathepsin D had a higher specificity constant (kcat/Km) than human cathepsin D. The stability of both enzymes was measured at 50[deg]C and half-lives of 55 and 3 min were derived for icefish and human cathepsin D, respectively.


http://www.sciencedirect.com/science/article/B6T21-47RSDDH-188/2/dec798cd2b55aadcb2047aec49f9454

NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) catalyzes the first step in the catabolic pathway of the prostaglandins. This enzyme oxidizes the 15-hydroxyl group of prostaglandins to produce 15-keto metabolites which are usually biologically inactive. In this study the cDNA for human placental 15-PGDH was expressed in Escherichia coli and the recombinant enzyme was purified to homogeneity and characterized. The N-terminus of the recombinant protein was sequenced and found to be identical with the known amino-acid sequence of 15-PGDH. Determinations of Km and Vax values for a number of the prostaglandins and NAD+ indicate that the recombinant enzyme does not appear to be kinetically different from the human placental enzyme. Site-directed mutagenesis was used to examine the importance of two residues which are highly conserved in the short-chain dehydrogenases which are known to be related to 15-PGDH. Tyrosine-151 was changed to phenylalanine and serine while lysine-155 was changed to glutamine and leucine. Western blot analysis indicated that the mutant and wild-
type proteins were expressed at the similar levels. However, all of the mutant proteins were found to be inactive. These results indicate that both tyrosine-151 and lysine-155 are required for 15-PGDH activity.


http://www.sciencedirect.com/science/article/B6T21-3XX6VSX-31/2/272caaac42310c24cf6f42c6df0c4962a

Factor XIIIa belongs to a family of ubiquitous transglutaminases, which catalyze formation of covalent bonds between the [epsil]-amino group of specific lysines and the [gamma]-carboxyl group of glutamines. Factor XIII is synthesized as a zymogen and after activation, it participates in both the coagulation and fibrinolytic mechanisms. Most transglutaminases are intracellular, but factor XIII is both intracellular and extracellular. The biosynthesis of extracellular (plasma) factor XIII, with the structure of a noncovalent heterotetramer, A2B2, is complex. Here, evidence is presented from PCR analysis and Northern blotting that mRNAs for both A and B subunits are present in the liver. The distribution of mRNA, specific for factor XIII subunits, in various human tissues was also analyzed. Among the tissues examined, the only signal for B subunit was found in the liver. For subunit A, the signal was observed in placenta, liver, kidney, lung, skeletal muscle and heart with varying intensities; in brain or pancreas there was no signal. With an immunoperoxidase method, factor XIII A subunit was identified in the PLC/PRF/5 cell line. By ELISA and reverse immunoblotting, with antibodies specific for the A-B complex, it was also shown that these cells produce and secrete factor XIII. From all of these results, we conclude that the liver is a source of plasma factor XIII, and that the complex A2B2 is secreted from these cells.


http://www.sciencedirect.com/science/article/B6T21-3X5GRHM-8/2/894a052d0590cb63209194738699675

Fusion proteins of rat cytochrome P4501A1 with maize ferredoxin I (Fd) and pea ferredoxin NADP+ reductase (FNR), the last electron transfer proteins of the photosynthetic channel in plant chloroplasts, were obtained by gene fusion in the yeast expression vector pAAH5N. The encoded fusion proteins P4501A1-Fd, P4501A1-FNR, P4501A1-Fd-FNR and P4501A1-FNR-Fd were produced in microsomes of the yeast Saccharomyces cerevisiae AH22. Enzymatic assays were carried out in vitro with the isolated microsomes. P4501A1-Fd-FNR and P4501A1-FNR-Fd were found to catalyze P450-monoxygenase activities towards 7-ethoxycoumarin and the herbicide chlortoluron. P4501A1-Fd-FNR was the most efficient enzyme as measured in vitro in ferricyanide and cytochrome c reductions, as well as P450-monoxygenase assays. Apparent Km and kcat of P4501A1-Fd-FNR were 70 [mu]M and 7800 min-1 for NADPH, 13.2 [mu]M and 51.1 min-1 for 7-ethoxycoumarin, and 21.3 [mu]M and 23.8 min-1 for the herbicide chlortoluron, respectively. Fd in P4501A1-Fd-FNR fusion enzyme was found to be a limiting factor compared to P4501A1 fused to the yeast NADPH-cytochrome P450 reductase, an artificial enzyme described previously. The efficiency of electron transfer in the P4501A1 fusion proteins and a possible in vivo molecular coupling of Fd and FNR with microsomal cytochrome P4501A1 produced in plant chloroplasts are discussed.

Transglutaminases (EC 2.3.2.13) catalyze an acyl-transfer reaction between peptide-bound glutamine residues and primary amines, including the [epsilon]-amino group of lysine residues in protein. Purified human erythrocyte transglutaminase was found to have another activity, i.e., GTP hydrolysis. Treatment of the enzyme with iodoacetamide, a cysteine-directed reagent, caused a 94% loss of TGase activity within 8 min, but no significant loss of GTPase activity. Cys-277, a known residue which is selectively modified by iodoacetamide, was replaced with Ser by site-directed mutagenesis to assess the role of the Cys-277 in the transglutaminase/GTPase activities. Wild-type cDNA, coding for human endothelial cell transglutaminase, and its C277S-mutated cDNA were cloned into a plasmid vector that contained a promotor from phage T7, and then expressed in Escherichia coli. The wild-type recombinant enzyme was indistinguishable from human erythrocyte transglutaminase in mobility on a SDS-polyacrylamide gel, immunoreactivity and catalytic activities for transglutaminase and GTPase. However, the recombinant enzyme was not blocked at the N-terminal alanine residue, as is the case in the naturally occurring erythrocyte enzyme. The C277S mutant enzyme showed no transglutaminase activity, but had Km and kcat values for GTPase activity that were comparable to those of wild-type recombinant and natural erythrocyte enzymes. These results demonstrate that Cys-277 is essential for transglutaminase activity but not for GTPase activity, and that N-terminal blocking of tissue-type transglutaminase is not critical for either transglutaminase or GTPase activities.


We isolated cDNA clones for cytochromes b561 from sheep and porcine adrenal medullae using the RT-PCR technique. Comparison of the deduced amino acid sequences of various species showed that there are two fully-conserved regions in this cytochrome. In addition, one methionyl and six histidyl residues (potential heme ligands) are fully-conserved. Based on a plausible structural model in which a polypeptide spans the vesicle membranes six times and holds two heme B molecules, the first conserved sequence (69ALLYVRVFR77) is located on the extravesicular side of an [alpha]-helical segment and the second one (120SLHSW124) is located in an intravesicular loop connecting two [alpha]-helical segments, respectively. Consideration of the relative locations of the fully-conserved sequences, and the methionyl and histidyl residues in the model led to a proposal that the first and second conserved sequences are likely to form the binding sites for extravesicular ascorbic acid and intravesicular semidehydroascorbic acid, respectively. A mild alkaline-treatment of purified bovine cytochrome b561 in oxidized state led to a specific loss of an electron-accepting ability from ascorbic acid for a half of the heme center, suggesting a distinct role for each of the two hemes.
p-Aminobenzoic acid (PABA), an essential component of the vitamin folic acid, is derived from the aromatic branch-point precursor chorismate in two steps. 4-Amino-4-deoxychorismate (ADC) synthase converts chorismate and glutamine to ADC and glutamate, and is composed of two subunits, PabA and PabB. While various experiments have suggested that PabA and PabB act as a complex, attempts to isolate the intact complex have failed. We report here the first successful copurification of PabA and PabB by gel filtration chromatography. The association of PabA and PabB is greatly enhanced by the presence of 5 mM glutamine, and by preincubation at 37°C. Conversely, the association is greatly reduced at cold temperatures. We also report the isolation and characterization of both chemically induced and site-directed mutations in PabB. Mutated PabB enzymes fall into three categories according to their properties: deficiency of chorismate amination coupled with failure to associate with PabA, deficiency of chorismate amination coupled with retention of PabA association, and competency of chorismate amination with failure of PabA association.

In this study we purified and investigated the catalytic properties of a manganese peroxidase isoenzyme produced by the fungus Pleurotus ostreatus in liquid medium with peptone as nitrogen source. The isoenzyme was purified to homogeneity by chromatography on Bio-Rad Q-cartridge, Sephacryl S-200 and Mono-Q with activity yield of 59% and a purification factor of 36. The P. ostreatus MnP obtained had the same pI (3.75) and N-terminal sequence as MnP-1 of Pleurotus eryngii produced in the same medium (both exhibiting Mn-independent activities on phenolic and non-phenolic substrates). However, the N-terminal sequence of this P. ostreatus isoenzyme differed from a previous published sequence of MnP from this fungus. The results obtained show the importance of media composition in the production of different isoenzymes within the same fungal species. We have also demonstrated by Southern blots that the different isoenzymes are probably encoded by different genes, and that the MnP genes in both Pleurotus species are similar but different to those of Phanerochaete chrysosporium.
determined by direct protein sequencing matched the amino acid sequence (453 residues) deduced from cloned HCC T-ALDH cDNAs with an open reading frame. The coding sequences of HCC T-ALDH cDNA, human stomach ALDH3A1 cDNA [Hsu et al., J. Biol. Chem. 267 (1992) 3030-3037] and human squamous cell carcinoma (SCC) T-ALDH cDNA (Schuuring et al., GenBank I.D. M74542) matched one another except for discrepancies at four positions, with consequent P12R, I27F and S134A substitutions. R and A were found in HCC and SCC T-ALDHs, whereas P and S were present in stomach ALDH3A1. To confirm that these discrepancies would have general occurrence, coding sequences of HCC T-ALDH cDNAs from six patients and stomach ALDH3A1 cDNAs from two individuals were examined and all were found to encode ALDH3A1 having R, I and A at protein positions 12, 27 and 134, respectively, indicating HCC T-ALDH to be variant ALDH3A1 which is common in human stomach tissues.


http://www.sciencedirect.com/science/article/B6T21-3V5 MPS1-N/2/ba8742d5feca96c5431bf608dbc99792

Although originally described in the male rodent genital tract, cysteine-rich secretory proteins (CRISPs) are expressed in a variety of mammalian tissue and cell types. The proteins of the male genital tract have been observed associated to spermatozoa and are believed to play a role in mammalian fertilization. Here we describe the identification and primary structure of the first equine member of the CRISP family. Equine CRISP-3 is transcribed and expressed in the stallion salivary gland, in the ampulla and the seminal vesicle. It displays all 16 conserved cysteine residues and shows 82% homology to human and 78% to guinea pig CRISP-2 (AA1, TPX 1) and 77% to human CRISP-3. In contrast to other mammals, in the horse CRISP-3 is synthesized in great amounts in the accessory sexual glands, ampulla and seminal vesicle, thus allowing the isolation of equine CRISP-3 in amounts suitable for biochemical, physiological and structural studies from stallion seminal plasma.


http://www.sciencedirect.com/science/article/B6T21-437XS03-P/2/d3c51317136776ecfafe3551292de247

We attempted to apply the directed evolution approach to enhancing enzyme properties in the presence of organic solvents, in which enzyme stability and activity were often drastically reduced. Stability and catalytic activity of phospholipase A1 in the presence of an organic solvent were enhanced by error-prone polymerase chain reaction (PCR) and DNA shuffling followed by a filter-based visual screening. Three mutants (SA8, SA17 and SA20) were isolated on indicator plates (i.e., 1% phosphatidylcholine gels containing 30% dimethyl sulfoxide (DMSO)) after a second mutant library was treated in 50% DMSO for 36 h. The half-life values of the three mutants exhibited an approximately 4-fold increase. The three mutants also exhibited increased stability in all organic solvents tested compared with the wild-type enzyme. Thus, an enzyme variant having superior catalytic efficiency in most of the organic solvents could be obtained by using any solvent suitable for designing the efficient screening system, regardless of the properties of the particular solvent.

A gene encoding a thermostable ascorbate oxidase (ASOM) was cloned from Acremonium sp. HI-25 and sequenced. The gene comprised 1709 bp and was interrupted by a single intron of 57 bp. ASOM consisted of 551 amino acids including a signal peptide with a molecular mass of 61200, and contained four histidine-rich regions with high sequence homology to the corresponding regions of other multicopper oxidases. The ASOM gene was expressed in Aspergillus nidulans under the Aspergillus oryzae Taka-amylase A gene promoter. The recombinant enzyme (An-ASOM) exhibited almost the same enzymatic properties as ASOM. The ASOM gene was mutated by site-directed mutagenesis with reference to the amino acid sequences of plant enzymes to generate enzymes with altered azide sensitivity. Site-directed mutagenesis at the trinuclear active copper site resulted in an increase in azide resistance; the Ala465Leu and Phe463Trp/Ala465Leu mutants exhibited approximately 10 and 20% increases in azide resistance, respectively.


Genes encoding 10914 Da and 58267 Da polypeptides homologous to groES and groEL of Escherichia coli were cloned and sequenced from a thermophilic cyanobacterium, Synechococcus vulcanus. The deduced amino acid sequence of the GroEL protein was much more homologous to GroELs of other cyanobacteria which accompany GroES than another GroEL homolog of S. vulcanus (GroEL2) reported previously (M. Furuki, N. Tanaka, T. Hiyama, and H. Nakamoto, Biochim. Biophys. Acta 1294 (1996) 106-110). We designate the gene as groEL1 to distinguish it from the non-operon forming groEL2 gene. A 9-base pair inverted repeat sequence (TTAGCAGTC-N9-GAGTGCTAA) was located upstream of the promoter region of groEL1, which was absent in groEL2. Southern blot analysis indicated that only one groESL1 operon was present in the genomic DNA of S. vulcanus. The amount of the bicistronic, 2.3 kb transcript of groESL1 operon increased 30-fold within 30 min upon heat shock. The increase was completely inhibited by chloramphenicol, suggesting the involvement of heat-induced production of a polypeptide. Introduction of the cloned groEL1 gene into a groEL defective mutant of E. coli resulted in the complementation of heat sensitivity, which contrasted with the previous result with groEL2.


http://www.sciencedirect.com/science/article/B6T21-3X1W5DP-F/2/22d620399c34caba2e06c652724c8d6a
By microsequencing and cDNA cloning we have identified the transformation-sensitive protein No. IEF SSP 9302 as the human homologue of calumenin. The nucleotide sequence predicts a 315 amino acid protein with high identity to murine and rat calumenin. The deduced protein contains a 19 amino acid N-terminal signal sequence, 7 EF-hand domains and, at the C-terminus, a HDEF sequence which has been reported to function as retrieval signal to the ER. The calumenin transcript is ubiquitously expressed in human tissue, at high levels in heart, placenta and skeletal muscle, at lower levels in lung, kidney and pancreas and at very low levels in brain and liver. Calumenin belongs to a family of multiple EF-hand proteins that include the ER localized proteins reticulocalbin and ERC-55 and the Golgi localized Cab45. Since its Ca2+ binding may be important for the function of the protein we have used microdialysis experiments in order to analyse for the affinity and the capacity of recombinant human (rh) calumenin. All 7 EF-hands of the protein are functional and bind Ca2+, each with an affinity of 1.6 x 103 M-1. The relatively low affinity for the EF-hands may suggest a role for the protein in Ca2+-dependent processes in the ER.


http://www.sciencedirect.com/science/article/B6T21-3VXBTBF-D/2/87bf131efab7232d08f2764e0789cf28

Protein kinase recognition sequences and proteinase sites were engineered into the cDNA encoding firefly luciferase from Photinus pyralis in order to establish whether these modified proteins could be developed as bioluminescent indicators of covalent modification of proteins. Two key domains of the luciferase were modified in order to identify regions of the protein in which peptide sequences may be engineered whilst retaining bioluminescent activity; one between amino acids 209 and 227 and the other at the C-terminus, between amino acids 537 and 550. Mutation of amino acids between residues 209 and 227 reduced bioluminescent activity to less than 1% of wild-type recombinant. In contrast engineering peptide sequences at the C-terminus resulted in specific activities ranging from 0.06-120% of the wild-type recombinant. Addition of cyclic AMP dependent protein kinase catalytic subunit, to a variant luciferase incorporating the kinase recognition sequence, LRRASLG, with a serine at amino-acid position 543 resulted in a 30% reduction in activity. Alkaline phosphatase treatment restored activity. The bioluminescent activity of a variant luciferase containing a thrombin recognition sequence, LVPRAS, with the cleavage site positioned between amino acid 542 and 543, decreased by 50% when incubated in the presence of thrombin. The results indicate regions within luciferase where peptide sequences may be engineered while retaining bioluminescent activity and have shown changes in bioluminescent activity when these sites are subjected to covalent modification. Changes in secondary structure, charge and length at the C-terminus of luciferase disrupt the microenvironment of the active site, leading to alterations in light emission. This has important implications both in understanding the evolution of beetle bioluminescence and also in the development of bioluminescent indicators of the covalent modification of proteins.

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Arcuri, C., I. Giambanco, et al. (2002). "Subcellular localization of S100A11 (S100C, calgizzarin) in
S100A11 is a member of a multigenic family of Ca2+-modulated proteins of the EF-hand type. We studied the subcellular localization of S100A11 in developing and adult avian skeletal muscle cells by confocal laser scanning microscopy and immunogold cytochemistry to get information about possible functional roles of this protein. Analyses of [alpha]-actinin, S100A1 and S100B were done in parallel for comparison. Low levels of S100A11 were found in skeletal muscle cells at embryonic day (E) 8. At E12, S100A11 was found in myotubes in the form of fine dots located between Z-discs, and on the sarcolemma and its invaginations. At E15, S100A11 was found on the sarcolemma and internal membranes, likely longitudinal tubules, where the protein was co-localized in part with S100A1 and S100B. At E18 and afterwards, co-localization of the three S100 proteins on internal membranes was almost complete. No evidence for association of S100A11 with the contractile elements of the sarcomeres was obtained. Our data suggests that, like S100A1 and S100B, S100A11 might have a role in the regulation of membrane activities, probably in relation to Ca2+ fluxes in skeletal muscle cells.

Mycobacterium tuberculosis is a major global pathogen whose threat has increased with the emergence of multidrug-resistant strains. The cell wall of M. tuberculosis is thick, rigid, and hydrophobic, which serves to protect the organism from the environment and makes it highly impermeable to conventional antimicrobial agents. There is little known about cell wall autolysins (also referred to as peptidoglycan hydrolases) of mycobacteria. We identified an open reading frame (Rv3915) in the M. tuberculosis genome designated cwlM that appeared consistent with a peptidoglycan hydrolase. The 1218-bp gene was amplified by PCR, cloned and expressed in E. coli strain HMS174(DE-3), and its gene product, a 47-kDa recombinant protein, was purified and partially characterized. Purified CwlM was able to lyse whole mycobacteria, release peptidoglycan from the cell wall of Micrococcus luteus and Mycobacterium smegmatis, and cleave N-acetylmuramoyl-L-alanyl-D-isoglutamine, releasing free N-acetylmuramic acid. These results indicate that CwlM is a novel autolysin and identify cwlM as the first, to our knowledge, autolysin gene identified and cloned from M. tuberculosis. CwlM offers a new target for a unique class of drugs that could alter the permeability of the mycobacterial cell wall and enhance the effectiveness of treatments for tuberculosis.
funiculosum xylanase, XYNC, with three known xylanase inhibitor proteins from wheat (Triticum aestivum). The xylanase gene (xynC) was cloned from a P. funiculosum genomic library and the deduced amino acid sequence of XYNC exhibited high sequence similarity with fungal family 11 xylanases. xynC was overexpressed in P. funiculosum and the product (XYNC: Mr=23.6 kDa; pI=3.7) purified and shown to efficiently degrade birchwood xylan [Km=0.47% w/v, Vmax=2540 [mu]mol xylose min-1 (mg protein)-1 at pH 5.5 and 30 [deg]C] and soluble wheat arabinoxylans [Km=1.45% w/v, Vmax=7190 [mu]mol xylose min-1 mg protein)-1 at pH 5.5 and 30 [deg]C]. The xylanase activity of XYNC was inhibited strongly by three xylanase inhibitor proteins from wheat; XIP-I, TAXI I and TAXI II. The inhibition for each was competitive, with very tight binding (Ki=3.4, 16 and 17 nM, respectively) equivalent to free energy changes ([Delta]G[deg]) of -49, -45 and -45 kJ mol-1. This is the first report describing a xylanase that is inhibited by all three wheat xylanase inhibitor proteins described to date.


http://www.sciencedirect.com/science/article/B73DJ-47X71D5-1/2/17b7a0e3e433d90bee0844bf5895a8c5

Gluconobacter strains effectively produce -sorbose from -sorbitol because of strong activity of the -sorbitol dehydrogenase (SLDH). -sorbose is one of the important intermediates in the industrial vitamin C production process. Two kinds of membrane-bound SLDHs, which consist of three subunits, were reportedly found in Gluconobacter strains [Agric. Biol. Chem. 46 (1982) 135,FEMS Microbiol. Lett. 125 (1995) 45]. We purified a one-subunit-type SLDH (80 kDa) from the membrane fraction of Gluconobacter suboxydans IFO 3255 solubilized with Triton X-100 in the presence of -sorbitol, but the cofactor could not be identified from the purified enzyme. The SLDH was active on mannitol, glycerol and other sugar alcohols as well as on -sorbitol to produce respective keto-aldoses. Then, the SLDH gene (sldA) was cloned and sequenced. It encodes the polypeptide of 740 residues, which contains a signal sequence of 24 residues. SLDH had 35-37% identity to those of membrane-bound quinoprotein glucose dehydrogenases (GDHs) from Escherichia coli, Gluconobacter oxydans and Acinetobacter calcoaceticus except the N-terminal hydrophobic region of GDH. Additionally, the sldB gene located just upstream of sldA was found to encode the polypeptide consisting of 126 very hydrophobic residues that is similar to the one-sixth N-terminal region of the GDH. Development of the SLDH activity in E. coli required co-expression of the sldA and sldB genes and the presence of PQQ. The sldA gene disruptant showed undetectable oxidation activities on -sorbitol in growing culture, and resting-cell reaction (pH 4.5 and 7); in addition, they showed undetectable activities on -mannitol and glycerol. The disruption of the sldB gene by a gene cassette with a downward promoter to express the sldA gene resulted in formation of a larger size of the SLDH protein and in undetectable oxidation of the polyols. In conclusion, the SLDH of the strain 3255 functions as the main polyol dehydrogenase in vivo. The sldB polypeptide possibly has a chaperone-like function to process the SLDH polypeptide into a mature and active form.


http://www.sciencedirect.com/science/article/B73DJ-4DB4YJK-2/2/a0356b1a6be6e8fd049f56c34919148d

The molecular mechanism of action of presynaptically toxic secreted phospholipases A2
(sPLA2s) isolated from snake venoms is not completely understood. It has been proposed that the positive charge in the [beta]-structure region is important for their toxic activity. To test this hypothesis, we characterised several mutants of ammodytoxin A (AtxA) possessing substitution of all five basic residues in this region. The mutations had relatively little influence on the catalytic activity of AtxA, either on charge-neutral or anionic phospholipid vesicles. An exception was R72 when replaced by a hydrophobic (higher activity) or an acidic (lower activity) residue. Lethal potencies of the eight single site mutants were up to four times lower than that of the wild-type, whereas the triple mutant (K74S/H76S/R77L) was 13-fold less toxic. The substitutions also lowered the affinity of the toxin, slightly to moderately, for the neuronal receptors R25 and R180. Interaction with calmodulin was only slightly affected by substitutions of K86, more by those of the K74/H76/R77 cluster and most by those of R72 (up to 11-fold lower binding affinity). The results clearly indicate that the basic amino acid residues in the [beta]-region of AtxA contribute to, but are not necessary for, its neurotoxic effect.


The eukaryotic Melanoplus sanguinipes entomopoxvirus (MsEPV) genome reveals a homologous sequence to eubacterial nicotinamide adenine dinucleotide (NAD+)-dependent DNA ligases [J. Virol. 73 (1999) 533]. This 522-amino acid open reading frame (ORF) contains all conserved nucleotidyl transferase motifs but lacks the zinc finger motif and BRCT domain found in conventional eubacterial NAD+ ligases. Nevertheless, cloned MsEPV ligase seals DNA nicks in a NAD+-dependent fashion, while adenosine 5'-monophosphate (ATP) cannot serve as an adenylation cofactor. The ligation activity of MsEPV ligase requires Mg2+ or Mn2+. MsEPV ligase seals sticky ends efficiently, but has little activity on 1-nucleotide gap or blunt-ended DNA substrates even in the presence of polyethylene glycol. In comparison, bacterial NAD+-dependent ligases seal blunt-ended DNA substrates in the presence of polyethylene glycol. MsEPV DNA ligase readily joins DNA nicks with mismatches at either side of the nick junction, except for mismatches at the nick junction containing an A base in the template strand (A/A, G/A, and C/A). MsEPV NAD+-dependent DNA ligase can join DNA probes on RNA templates, a unique property that distinguishes this enzyme from other conventional bacterial NAD+ DNA ligases. T4 ATP-dependent DNA ligase shows no detectable mismatch ligation at the 3' side of the nick but substantial 5' T/G mismatch ligation on an RNA template. In contrast, MsEPV ligase joins mismatches at the 3' side of the nick more frequently than at the 5' side of the nick on an RNA template. The complementary specificities of these two enzymes suggest alternative primer design for genomic profiling approaches that use allele-specific detection directly from RNA transcripts.


http://www.sciencedirect.com/science/article/B73DJ-46DP2XD-2/2/a5c3309e825d18913817b4c35bab754f

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (EC 2.4.2.8) is an important enzyme involved in the recycling of purine nucleotides in all cells. Parasitic protozoa of the order Kinetoplastida are unable to synthesize purines de novo and use the salvage pathway for the
synthesis of nucleotides; therefore, this pathway is an attractive target for antiparasitic drug design. The hgprt gene was cloned from a Leishmania tarentolae genomic library and the sequence determined. The L. tarentolae hgprt gene contains a 633-nucleotide open reading frame that encodes a 23.4-kDa protein. A pairwise alignment of the different HGPRT's sequences revealed a 26%-53% sequence identity with the Leishmania sequences and 87% identity to the HGPRT of Leishmania donovani. A recombinant protein was expressed in Escherichia coli, purified to homogeneity and found to retain enzymatic activity. The steady-state kinetic parameters were determined for the recombinant enzyme and the enzyme is active as a homodimer in solution. Single crystals were obtained for the L. tarentolae HGPRT representing the first Leishmania HGPRT crystallized and initial crystallographic data were collected. The crystals obtained belong to the orthorhombic space group (P212121) with unit cell parameters a=58.104 Å, b=85.443 Å and c=87.598 Å and diffract to a resolution of 2.3 Å. The availability of the HGPRT enzyme from Leishmania and its crystallization suitable for X-ray diffraction data collection should provide the basis for a functional and structural analysis of this enzyme, which has been proposed as a potential target for rational drug design, in a Leishmania model system.


http://www.sciencedirect.com/science/article/B73DJ-49MD4SV-1/2/7f7c716ac41ae5b1b48e735777cedd0

The three-dimensional structure of Leishmania tarentolae adenine phosphoribosyltransferase (APRT) in complex with adenosine-5-monophosphate (AMP) and a phosphate ion has been solved. Refinement against X-ray diffraction data extending to 2.2-A resolution led to a final crystallographic R factor of 18.3%. Structural comparisons amongst this APRT enzyme and other 'type I' PRTases whose structures have been determined reveal several important features of the PRTases catalytic mechanism. Based on structural superpositions and molecular interaction potential calculations, it was possible to suggest that the PRPP is the first substrate to bind, while the AMP is the last product to leave the active site, in accordance to recent kinetic studies performed with the Leishmania donovani APRT.

Biochimie (3)


http://www.sciencedirect.com/science/article/B6VRJ-4DS4255-2/2/c5c38402b9b27dc4e6fd6b95f9f4d2b

In the human nuclear genome only a few copies coding for full-length 7SL RNA genes exist. The Hs7SL-1 gene has recently been classified as type 4 of RNA polymerase III (pol III)-transcribed genes as it was demonstrated that mutations in an external transcriptional activator (ATF) binding site and in an internal CG dinucleotide at positions +15/+16 reduced 7SL RNA expression in vivo and in vitro. We have extended the elucidation of external and internal promoter elements and have discovered two novel regulatory sequences: a TATA-like element in the upstream region
and internal A and B box-like motifs. This study was greatly facilitated by the identification of a second, new functional human 7SL RNA gene which we called Hs7SL-3. Remarkably, Hs7SL-3 RNA is synthesized twice as efficiently as Hs7SL-1 in HeLa nuclear extract. Comparison of the upstream regions revealed the presence of two conserved elements in the two human 7SL RNA genes, an ATF/CRE binding site at -43 to -50 and a TATA-like box centered around position -25. Mutational analyses indicated that both external promoter elements are important for efficient transcription. In addition, two sequence motifs can be identified in Hs7SL-1 and Hs7SL-3 at positions 10-19 and 50-60, respectively, downstream of the transcription start site that resemble putative A and B boxes. Single and multiple nucleotide substitutions in these regions also influenced transcription activity to a great extent. The requirement of intragenic functional A and B boxes in combination with the external ATF/CRE and TATA-like promoter elements for the efficient transcription of human 7SL RNA genes is reminiscent of at least two other classes of pol III-transcribed genes in human cells, such as Epstein-Barr virus-encoded EBER and vault RNA genes.


http://www.sciencedirect.com/science/article/B6VRJ-3Y6Y729-2/2/6b222932427205f69bca308b1da671db

Here we report the cloning of a cDNA encoding the first nucleoside diphosphate kinase (NDPK) isolated from plant mitochondria. Amplification of a 317 nt product was performed by PCR, using oligonucleotide primers based on partial amino acid sequences of the pea mitochondria NDPK and other NDPK isoforms. By screening of a pea cDNA library with this PCR product, a full length clone was obtained. Northern analysis revealed the presence of a 1.1 kb single transcript, with high expression in young leaves and reproductive tissues. The clone encodes a precursor protein of 232 amino acids (26 kDa), including an N-terminal extension of 80 amino acids (9 kDa). Analysis of the deduced amino acid sequence confirmed its identity with the sequences obtained from the purified mature pea mitochondrial NDPK. In vitro import experiments carried out in isolated pea mitochondria showed targeting and processing of the 27 kDa precursor into a 16.5 kDa mature form. Phylogenetic analysis of some vertebrate and plant isoforms of NDPK showed that the pea mtNDPK groups together with the NDPK3 isoform from A. thaliana and the chloroplastic NDPK III from spinach. We suggest that it is possible to design a novel classification of the different NDPK isoforms according to their subcellular localisation and origin.


http://www.sciencedirect.com/science/article/B6VRJ-4F56CP-1/2/fe385b093c09be8fa92ef8e3e24c4a07

Lactose-binding proteins with molecular masses of 14-, 17-, 18-, 28-, and 34-kDa were identified in extracts from porcine small intestinal mucosa. Amino acid sequence analysis of peptides generated by CNBr cleavage of the 34-kDa protein, the most abundant of these proteins, identified this protein as porcine galectin-4. To determine if a porcine homolog of murine galectin-6 is expressed in small intestine, primers for a reverse transcriptase-polymerase chain reaction (RT-PCR) were developed that amplified across the linker region of galectin-4, which is the region that differs between murine galectins-4 and -6. Using these primers, this RT-PCR approach identified two galectin-4 isoforms that differed in the length of their linker region. The larger isoform, galectin-4.1, is nine amino acids longer in its linker region than the smaller isoform, galectin-4.2. Based on nucleotide sequence similarities, the two isoforms are likely splice variants
of galectin-4 pre-mRNA and not products of separate genes like murine galectins-4 and -6.

**Biol Reprod** (43)


http://www.biolreprod.org/cgi/content/abstract/70/4/1106

Although studies in transgenic mice suggest that estrogen is important for development of the testis, very little is known about the potential role of estrogen in maturation of the primate fetal testis. Therefore, as a first step to determine whether estrogen regulates maturation of the fetal primate testis, we used immunocytochemistry to determine estrogen receptor (ER) \{alpha\} and \{beta\} expression in the fetal baboon testis. Second, we established methods to quantify ER{beta} mRNA levels by competitive reverse transcription-polymerase chain reaction in Sertoli cells isolated by laser capture microdissection (LCM) from the fetal baboon testis. ER{beta} protein expression was abundant in the nuclei of Sertoli, peritubular, and interstitial cells in baboon fetuses at mid (Day 100) and late (Day 165) gestation (term is 184 days). ER{beta} mRNA level was 0.03 attomole/femtomole 18S rRNA in Sertoli cell nuclei and associated cytoplasm isolated by LCM. ER{alpha} was expressed in low level in seminiferous tubules and in moderate level in peritubular cells on Day 165. Germ cells expressed very little ER{alpha} or ER{beta} protein, whereas the baboon fetal epididymis exhibited extensive ER{alpha} and ER{beta} immunostaining at mid- and late gestation. In contrast to the robust expression of ER{beta}, androgen receptor protein was not demonstrable within the cells of the seminiferous cords but was abundantly expressed in epididymal epithelial cells of the fetal baboon. In summary, the results of this study show that the fetal baboon testis and epididymis expressed the ER{alpha} and ER{beta}, and we suggest that our nonhuman primate baboon model can be used to study the potential role of estrogen on maturation of the fetal testis.


http://www.biolreprod.org/cgi/content/abstract/71/3/820

In mammals, testis determination is initiated when the SRY gene is expressed in pre-Sertoli cells of the undifferentiated genital ridge. SRY directs the differentiation of these cells into Sertoli cells and initiates the testis differentiation pathway via currently ill-defined mechanisms. Because Sertoli cells are the first somatic cells to differentiate within the developing testis, it is likely that the signals for orchestrating testis determination are expressed within pre-Sertoli cells. We have previously generated a transgenic mouse line that expresses green fluorescent protein under the control of the pig SRY promoter, thus marking pre-Sertoli cells via fluorescence. We have now used suppression-subtractive hybridization (SSH) to construct a normalized cDNA library derived from fluorescence-activated cell sorting (FACS) purified pre-Sertoli cells taken from 12.0 to 12.5 days postcoitum (dpc) fetal transgenic mouse testes. A total of 35 candidate cDNAs for known genes were identified. Detection of Sf1, a gene known for its role in sex determination as well as Vanin-1, Vcp1, Sparc, and Aldh3a1, four genes previously identified in differential screens as
gene overexpressed in developing testis compared with ovary, support the biological validity of our experimental model. Whole-mount in situ hybridization was performed on the 35 candidate genes for qualitative differential expression between male and female genital ridges; six were upregulated in the testis and one was upregulated in the ovary. The expression pattern of two genes, Ppt1 and Brd3, were examined in further detail. We conclude that combining transgenically marked fluorescent cell populations with differential expression screening is useful for cell expression profiling in developmental systems such as sex determination and differentiation.


http://www.biolreprod.org/cgi/content/abstract/70/2/297

Androgens are known to attenuate some effects of estradiol-17\(\beta\) (E) in the uterus. The objectives of the present experiment were to determine effects of 5(\alpha\)-dihydrotestosterone (DHT) on estrogenic actions in the pig uterus and its associations with changes in expression of the estrogen receptor (ER) (\alpha) and ER(\beta). Postpubertal gilts (120-130 kg of body weight; \(n = 16\)) were ovariectomized, and 3-4 weeks later received once-a-day injections (i.m.) of one of the following treatments during four consecutive days: 1) vehicle (corn oil), 2) E (250 \(\mu\)g), 3) E (250 \(\mu\)g) plus 1 mg DHT, or 4) E (250 \(\mu\)g) plus 10 mg DHT. Uterine tissues were collected 24 h after the last treatment. Gilts receiving E or E plus 1 mg DHT had greater uterine wet weight, uterine horn diameter, luminal epithelium thickness, and endometrial gland diameter compared with gilts treated with vehicle or E plus 10 mg DHT. Gilts receiving E or E plus 1 mg DHT did not differ in these characteristics. Relative amounts of mRNAs in the endometrium for the cell proliferation marker histone H2a and the E-inducible protein complement component C3 in gilts treated with E compared with gilts treated with vehicle. E-induced increases in histone H2a and C3 mRNAs were not altered by cotreatment with E plus 1 mg DHT but were inhibited by E plus 10 mg DHT. Androgen receptor (AR) mRNA in the endometrium increased by treatment with E. Cotreatment of gilts with E and DHT did not alter the E-induced AR mRNA increase. Gilts treated with E plus 10 mg DHT had lesser amounts of immunoreactive ER(\alpha) in cell nuclei of the myometrium and endometrial stroma and a tendency for a decrease in luminal epithelium compared with gilts treated with E. Amounts of immunoreactive ER(\alpha) in glandular epithelium were not influenced by the treatments. Relative amounts of ER(\alpha) and ER(\beta) mRNAs decreased in the endometrium of gilts treated with E plus 10 mg DHT compared with gilts treated with E. Downregulation of the ERs, particularly ER(\alpha) in the myometrium and endometrial stroma, might be a relevant mechanism in the antagonism of estrogenic effects by DHT in the pig uterus.


http://www.biolreprod.org/cgi/content/abstract/69/3/851

Postnatal development of the ovine uterus between birth and Postnatal Day (PND) 56 involves differentiation of the endometrial glandular epithelium from the luminal epithelium followed by tubulogenesis and branching morphogenesis. Previous results indicated that ovariectomy of ewes at birth did not affect uterine growth or initial stages of endometrial gland genesis on PND 14 but did affect uterine growth after PND 28. Available evidence from a number of species supports the hypothesis that the ovary does not affect endometrial gland morphogenesis in the postnatal uterus. To test this hypothesis in our sheep model, ewes were assigned at birth to a sham
surgery as a control or bilateral ovariectomy (OVX) on PND 7. Uteri were removed and weighed on PND 56. Ovariectomy did not affect circulating levels of estradiol-17{beta}. Uterine weight was 52% lower in OVX ewes. Histomorphological analyses indicated that the thickness of the endometrium and myometrium, total number of endometrial glands, and endometrial gland density in the stratum spongiosum stroma was reduced in uteri of OVX ewes. In contrast, the number of superficial ductal gland invaginations and gland density in the stratum compactum stroma was not affected by ovariectomy. The uteri of OVX ewes contained lower levels of {beta}A subunit, activin receptor (ActR) type IA, ActRIB, and follistatin protein expression but higher levels of {beta}B subunit. In the neonatal ovary, follistatin, inhibin {alpha} subunit, {beta}A subunit, and {beta}B subunit were expressed in antral follicles between PNDs 0 and 56. These results led to rejection of the hypothesis that the ovary does not influence endometrial adenogenesis. Rather, the ovary and, thus, an ovarian-derived factor regulates, in part, the coiling and branching morphogenetic stage of endometrial gland development after PND 14 and expression of specific components of the activin-follistatin system in the neonatal ovine uterus that appear to be important for that critical process.


http://www.biolreprod.org/cgi/content/abstract/biolreprod.105.039776v1

The ovine genome contains approximately 20 copies of endogenous betaretroviruses (enJSRVs) that are highly related to two exogenous oncogenic viruses, Jaagsiekte sheep retrovirus (JSRV) and Enzootic nasal tumor virus. The cellular receptor for both JSRV and the enJSRVs is hyaluronidase 2 (HYAL2). In this study, we assessed expression of enJSRVs envelope (env) and HYAL2 mRNAs in the ovine uterus and conceptus (embryo/fetus and extraembryonic membranes) throughout gestation. By RT-PCR analyses, enJSRVs env were found to be expressed beginning in the Day 12 conceptus, whereas HYAL2 was expressed from Day 16. HYAL2 mRNA was detected throughout gestation in the placentome, but not in the endometrium, whereas enJSRVs env expression was detected throughout gestation in endometrium and placentomes. The enJSRVs env mRNA was specifically expressed in the endometrial lumenal epithelium (LE) and glandular epithelium (GE) as well as the trophoblast giant binucleate cells (BNC) and multinucleated syncytiotrophoblast of the placenta. HYAL2 mRNA was only detected in the BNC and multinucleated syncytial plaques of the placentome. Partial sequencing of the transcriptionally active enJSRVs from sheep endometrium, placentomes and placenta revealed expression of many enJSRV loci. Cloning of the expressed enJSRVs env mRNA from ovine uteroplacental tissues found sequences similar to the previously identified enJS5F16 and enJS56A1 gene with an intact open reading frame, although the polypeptides they encode were not studied. Collectively, results provide further support for our hypothesis that the enJSRVs Env have been beneficial to the host and are involved in protection of the uterus from viral infection and regulators of placental morphogenesis and function.


http://www.biolreprod.org/cgi/content/abstract/66/5/1456

Apoptosis induced in male germ cells following ionizing radiation is dependent on functional p53 (Trp53) being present. We sought to determine whether Fas (Tnfrsf6/CD95/APO-1), an apoptotic factor, is involved in this p53-dependent germ cell death. In p53 knock-out mice exposed to 5 Gy of x-radiation, germ cells were protected from cell death, as assessed by counting apoptotic
seminiferous tubules 12 h following radiation. Similarly, spermatid head counts in p53 knock-out mice remained near normal 29 days after exposure to 0.5 Gy of radiation, whereas wild-type animals had a more than twofold reduction in spermatid head counts. Fas mRNA expression remained at pretreatment levels in p53 knock-out mice; however, Fas increased in a time-dependent manner in wild-type mice following exposure to 5 Gy of radiation, indicating that radiation-induced Fas expression is p53-dependent. The functional significance of Fas involvement was demonstrated when lprcg mice, having a non-functional Fas receptor, were exposed to 5 Gy of radiation; the number of apoptotic seminiferous tubules 12 h following radiation was significantly reduced compared to that of wild-type mice. Additionally, lprcg mice exposed to 0.5 Gy of radiation had increased spermatid head counts 29 days following radiation compared to wild-type mice. Interestingly, gld mice with a non-functional Fas ligand (Tnfsf6/FasL/CD95L) were as sensitive to radiation as wild-type animals, and levels of FasL mRNA were not affected by radiation treatment. These results indicate that apoptosis and up-regulation of Fas following radiation are both p53-dependent events. Although Fas is necessary, in part, for radiation-induced p53-dependent apoptosis, FasL is not.


http://www.biolreprod.org/cgi/content/abstract/70/2/523

Development of antral follicles beyond 3 to 4 mm in cattle appears as a wave pattern that occurs two to three times during the estrous cycle. Each wave presents a cyclic recruitment of multiple follicles at the 3- to 4-mm stage, followed by the selection of a single follicle that becomes the dominant follicle (DF). The molecular determinants involved in the follicular dominance process remain poorly understood. The objective of the current study was to compare gene expression in granulosa cells (GCs) between growing dominant follicles from Day 5 of the estrous cycle and nonselected small follicles (<=4 mm) using the suppression subtractive hybridization (SSH) approach to identify candidate genes differentially expressed in GCs of the DF. Small follicle cDNAs were subtracted from DF cDNAs (DF-SF) and used to establish a DF GC-subtracted cDNA library. A total of 42 nonredundant cDNAs were identified. Detection of previously identified genes such as CX43, CYP19, INHBA, and SERPINE2 supported the validity of our experimental model and the use of SSH as the method of analysis. For selected genes such as ApoER2, CPD, CSPG2, 14-3-3 epsilon, NR5A2/SF2, RGN/SMP30, and SERPINE2, gene expression profiles were compared by virtual Northern blot or reverse transcriptase-polymerase chain reaction, and results confirmed an increase or induction of their mRNA in GCs of dominant follicles compared with that of small follicles. We conclude that we have identified novel genes (known and unknown) that are up-regulated in bovine GCs that may affect follicular growth, dominance, or both.


http://www.biolreprod.org/cgi/content/abstract/72/2/470

The prostate gland in the brushtail possum grows and regresses seasonally. It has similarities to the human prostate and may therefore provide a unique model for investigating prostatic hyperplasia. Oxytocin has been implicated in the regulation of prostate growth in eutherian mammals, and the initial aim of this study was to identify and localize the marsupial equivalent, mesotocin, and its receptor in the prostate of the brushtail possum. Seasonal changes in prostatic mesotocin concentrations and receptor localization were then assessed and related to prostate
growth. Mesotocin and mesotocin receptor gene transcripts with high sequence homology to eutherian oxytocin/oxytocin receptors were demonstrated, and mesotocin, neurophysin, and the receptor were all localized predominantly in the epithelial cells of the glandular acini. Western blot analysis confirmed the presence of a single immunoreactive receptor protein of ~60 Mr-3. Prostatic mesotocin concentrations were highest immediately before the increases in prostate weight associated with the autumn and spring breeding periods. At this time, mesotocin receptors were also present in the prostatic capsule in addition to those present in the glandular tissue. Mesotocin concentrations proceeded to decrease in association with the regression of prostate size toward the end of the breeding periods. No significant differences were present in serum testosterone or dihydrotestosterone throughout the year. The identification of mesotocin and its receptor in the possum prostate and the demonstration of seasonal changes in local mesotocin concentrations preceding changes in prostate size suggests that mesotocin may play a physiological role in regulating prostate growth and regression.


http://www.biolreprod.org/cgi/content/abstract/66/2/524

This study identified prostaglandin D2 synthase (PGDS) in murine epididymal fluid using a proteomic approach combining two-dimensional (2D) gel electrophoresis and mass spectrometry (MS). The caudal epididymal fluid was collected by retroperfusion, and proteins were separated by 2D gel electrophoresis followed by matrix-assisted laser desorption ionization MS analyses after trypsin digestion. The identification was based on the protein-specific peptide map as well as on sequence information generated by nano-electrospray ionization MS/MS. By in situ hybridization, the mRNA was detected in caput, corpus, and cauda, but it was not detected in the initial segment. The PGDS protein was mostly detected in the corpus and cauda by Western blot analysis and immunohistochemistry using a specific polyclonal antibody. In caudal fluid, PGDS was distributed among several isoforms (pI range, 6.5-8.8), suggesting that this protein undergoes posttranslational modification of its primary sequence. After N-glycanase digestion, the molecular mass decreased from 20-25 to 18.5 kDa, its theoretical mass. The PGDS was also detected in the epididymis of rat, hamster, and cynomolgus monkey from the caput to the cauda. In conclusion, MS is a powerful and accurate technique that allows unambiguous identification of the murine epididymal PGDS. The protein is 1) present throughout the epididymis, except in the initial segment, with an increasing luminal concentration from distal caput to cauda; 2) a major protein in caudal fluid; 3) an N-glycosylated, highly polymorphic protein; and 4) conserved during evolution.


http://www.biolreprod.org/cgi/content/abstract/69/5/1665

Peroxisome proliferator-activated receptor (PPAR{gamma}) is a nuclear receptor that is activated by fatty acids and derivatives and the antidiabetic glitazones, which plays a role in the control of lipid and glucose homeostasis. In the present work, we tested the hypothesis that PPAR{gamma} plays a role in reproductive tissues by studying its expression and function in the hypothalamo-pituitary-ovary axis in the sheep. PPAR{gamma} 1 and PPAR{gamma} 2 proteins and mRNAs were detected in whole ovine pituitary and ovary but not in hypothalamic extracts. In situ hybridization on ovarian section localized PPAR{gamma} mRNA in the granulosa layer of follicles.
Interestingly, PPAR\(_{\gamma}\) expression was higher in small antral (1-3 mm diameter) than in preovulatory follicles (>5 mm diameter) (P < 0.001) and was not correlated with healthy status. To assess the biological activity of ovarian PPAR\(_{\gamma}\), ovine granulosa cells were transfected with a reporter construct driven by PPAR\(_{\gamma}\)-responsive elements. Addition of rosiglitazone, a PPAR\(_{\gamma}\) ligand, stimulated reporter gene expression, showing that endogenous PPAR\(_{\gamma}\) is functional in ovine granulosa cells in vitro. Moreover, rosiglitazone inhibited granulosa cell proliferation (P < 0.05) and increased the secretion of progesterone in vitro (P < 0.05). This stimulation effect was stronger in granulosa cells from small than from large follicles. In contrast, rosiglitazone had no effect on LH, FSH, prolactin and growth hormone secretion by ovine pituitary cells in vitro. Overall, these data suggest that PPAR\(_{\gamma}\) ligands might stimulate follicular differentiation in vivo likely through a direct action on granulosa cells rather than by modulating pituitary hormone secretion.


http://www.biolreprod.org/cgi/content/abstract/71/1/89

Jak2, which is a member of the Janus tyrosine kinase family, plays essential roles in cytokine signal transduction and in the regulation of cell growth and gene expression. To investigate the involvement of Jak2 in the regulation of early preimplantation development, we examined the expression of Jak2 in mouse embryos. Reverse transcription-polymerase chain reaction assays revealed that the relative amount of Jak2 mRNA was highest in unfertilized oocytes, gradually decreased until the four-cell stage, and remained at low levels until the blastocyst stage. Immunocytochemistry showed that Jak2 was localized predominantly to the female pronucleus in one-cell embryos. The immunofluorescence signal was very weak or undetectable in the male pronucleus. In unfertilized oocytes and one-cell embryos at M phase, Jak2 was localized to the chromosomes. After cleavage to the two-cell stage, the intensity of the immunofluorescence signal decreased in the nucleus while the embryos were in late G2. This decrease was independent of DNA synthesis because it was not affected by inhibition of DNA replication. However, inhibition of protein synthesis repressed the disappearance of Jak2 from the nucleus. These results suggest a novel function for Jak2 in the regulation of early preimplantation development.


http://www.biolreprod.org/cgi/content/abstract/67/6/1981

In mice deficient in progesterone receptor (PR), follicles of ovulatory size develop but fail to ovulate, providing evidence for an essential role for progesterone and PR in ovulation in mice. However, little is known about the expression and regulation of PR mRNA in preovulatory follicles of ruminant species. One objective of this study was to determine whether and when PR mRNA is expressed in bovine follicular cells during the periovulatory period. Luteolysis and the LH/FSH surge were induced with prostaglandin F2\(\alpha\) and a GnRH analogue, respectively, and the preovulatory follicle was obtained at 0, 3.5, 6, 12, 18, or 24 h after GnRH treatment. RNase protection assays revealed a transient increase in levels of PR mRNA, which peaked at 6 h after GnRH and declined to the time 0 value by 12 h and a second increase at 24 h. The second objective was to investigate the mechanisms that regulate PR mRNA expression through in vitro studies on follicular cells of preovulatory follicles obtained before the LH/FSH surge. Theca and granulosa cells were isolated and cultured with or without a luteinizing dose of LH or FSH, progesterone, LH + progesterone, or LH + antiprogestin (RU486). Levels of PR mRNA increased
in a time-dependent manner in granulosa cells cultured with LH or FSH and in theca cells cultured with LH, peaking at 10 h of culture. In contrast, progesterone (200 ng/ml) did not upregulate mRNA for its own receptor, and neither progesterone nor RU486 affected LH-stimulated PR mRNA accumulation. Furthermore, RU486 completely blocked LH-stimulated expression of oxytocin mRNA, indicating that PR induced by LH in vitro is functional. These results show that the gonadotropin surge induces a rapid and transient increase in expression of PR mRNA in both theca and granulosa cells of bovine periovulatory follicles followed by a second rise close to the time of ovulation and that the first increase in PR mRNA can be mimicked in vitro by gonadotropins but not by progesterone. These results suggest multiple and time-dependent roles for progesterone and PR in the regulation of periovulatory events in cattle.


Germ cell transplantation is a technique that transfers donor testicular cells into recipient testes. A population of germ cells can colonize the recipient testis, initiate spermatogenesis, and produce sperm capable of fertilization. In the present study, a nonmosaic Klinefelter bull was used as a germ cell recipient. The donor cell suspension was introduced into the rete testis using ultrasound-guided puncture. A pulsatile administration of GnRH was performed to stimulate spermatogenesis. The molecular approach to detect donor cells was done by a quantitative polymerase chain reaction with allele discrimination based on a genetic mutation between donor and recipient. Therefore, a known genetic mutation, associated with coat-color phenotype, was used to calculate the ratio of donor to recipient cells in the biopsy specimens and ejaculates for 10 mo. After slaughtering, meiotic preparations were performed. The injected germ cells did not undergo spermatogenesis. Six months after germ cell transplantation, the donor cells were rejected, which indicates that the donor cells could not incorporate in the testis. The hormone stimulation showed that the testosterone-producing Leydig cells were functionally intact. Despite subfertility therapy, neither the recipient nor the donor cells underwent spermatogenesis. Therefore, nonmosaic Klinefelter bulls are not suitable as germ cell recipients. Future germ cell recipients in cattle could be mosaic Klinefelters, interspecies hybrids, bulls with Sertoli cell-only syndrome, or bulls with disrupted germ cell migration caused by RNA interference.


The transient synthesis and accumulation of hyaluronan (HA), an extracellular matrix component of cumulus cells, brings about expansion of cumulus-oocyte complexes (COCs) in preovulatory mammalian follicles. In this study, we investigated the mRNA expressions of hyaluronan synthase 2 (has2), hyaluronan synthase 3 (has3), and CD44, as well as the responsiveness to eCG and porcine follicular fluid (pFF) of these genes, in porcine COCs, oocyteectomized complexes (OXC), and oocytes during in vitro maturation. Immunolocalization of CD44 was also analyzed in COCs. After 12 h of culture, the area of cumulus expansion in medium 199 supplemented with both 10 IU/ml eCG and 10% (v/v) pFF was significantly greater than that in the medium supplemented with eCG or pFF. Oocytectomy reduced the expansion area in the group supplemented with eCG. In reverse transcription-polymerase chain reaction analysis, all transcripts were identified in COCs, but has3 transcript was not found in OXC. Only has3 mRNA was detectable in oocytes, indicating that cumulus cells express has2 and CD44 mRNAs, and oocytes express has3 mRNA. The expression levels of has2 and CD44 mRNAs in COCs and
OXCs increased in the presence of eCG and pFF after 24 h of culture, suggesting that these genes have a positive dependency on eCG and pFF. In contrast, the high level of has3 mRNA was detected in COCs cultured in the medium alone. Oocytectomy slightly reduced the expression level of has2 mRNA. On immunostaining for CD44, CD44 was expressed apparently in COCs cultured with eCG and pFF for 24 h. The positive staining was distributed on cytoplasm along the perimembrane of cumulus cells and at the junctions between cumulus cells and oocytes. CD44 was also localized on cytoplasm of some oocytes. These results indicate that 1) porcine oocytes promote eCG-dependent cumulus expansion and the expression of has2 mRNA in cumulus cells, but these are not essential for expansion of cumulus cells and the expression of has2 mRNA; 2) HAS2 is involved in HA synthesis during cumulus expansion, and eCG and pFF up-regulate its expression; 3) the expression profile of the has3 mRNA that is transcribed in oocytes is different from those of has2 and CD44 mRNA; and 4) CD44 may participate in the interaction between cumulus cells and oocytes.


http://www.biolreprod.org/cgi/content/abstract/69/1/261

Translational control of specific mRNAs by cytoplasmic polyadenylation has fundamental roles in gametogenesis. The cytoplasmic polyadenylation element binding (CPEB) protein regulates cytoplasmic polyadenylation of mRNAs as a trans factor in oogenesis and spermatogenesis. The CPEB protein contains two RNA recognition motifs and a Zn-finger structure. Proteins (KIAA0940 and KIAA1673) with similar structures are predicted from the genome database, but nothing is known about their expression and function. Here, we report another novel member of the CPEB protein family, CPEB2. Comparison of the amino acid sequences of CPEB family members suggests that the family can be divided structurally and, perhaps, functionally into two groups: the CPEB group, and the CPEB2-KIAA0940-KIAA1673 group. The CPEB2 maps to mouse chromosome distal 5B and is abundantly expressed in testis. However, it was detected by reverse transcription-polymerase chain reaction in all tissues that we examined. It preferentially binds to poly(U) and localizes to the cytoplasm in transfected HeLa cells. The CPEB2 is expressed postmeiotically in mouse spermatogenesis, suggesting a possible role in translational regulation of stored mRNAs in transcriptionally inactive haploid spermatids.


http://www.biolreprod.org/cgi/content/abstract/71/2/515

The ubiquitin pathway functions in the process of protein turnover in eukaryotic cells. This pathway comprises the enzymes that ubiquitinate/deubiquitinate target proteins and the proteasome that degrades ubiquitin-conjugated proteins. Ubiquitin C-terminal hydrolases (UCHs) are thought to be essential for maintaining ubiquitination activity by releasing ubiquitin (Ub) from its substrates. Mammalian UCH-L1 and UCH-L3 are small proteins that share considerable homology at the amino acid level. Both of these UCHs are highly expressed in the testis/ovary and neuronal cells. Our previous work demonstrated that UCH-L1-deficient gracile axonal dystrophy (gad) mice exhibit progressively decreasing spermatogonial stem cell proliferation, suggesting that UCH isozymes in the testis function during spermatogenesis. To analyze the expression patterns of UCH isozymes during spermatogenesis, we isolated nearly homogeneous populations of spermatogonia, spermatocytes, spermatids, and Sertoli cells from mouse testes. Western blot analysis detected UCH-L1 in spermatogonia and Sertoli cells, whereas UCH-L3 was detected in spermatocytes and spermatids. Moreover, reverse transcription-polymerase chain
reaction analysis of UCH isozymes showed that UCH-L1 and UCH-L4 mRNAs are expressed in spermatogonia, whereas UCH-L3 and UCH-L5 mRNAs are expressed mainly in spermatocytes and spermatids. These results suggest that UCH-L1 and UCH-L3 have distinct functions during spermatogenesis, namely, that UCH-L1 may act during mitotic proliferation of spermatogonial stem cells whereas UCH-L3 may function in the meiotic differentiation of spermatocytes into spermatids.


http://www.biolreprod.org/cgi/content/abstract/68/5/1870

In this study, we examined the localization of vascular endothelial growth factor (VEGF) and the changes in VEGF mRNA expression in various regions of the oviduct in fertile women throughout the ovulatory cycle. Oviduct tissue was collected from 22 women undergoing laparoscopic tubal sterilization or hysterectomy for a benign gynecological condition. Oviduct sections were divided into isthmus, ampullary, and infundibular regions. Serial cross sections were analyzed for the presence of VEGF by specific immunohistochemical staining. The mucosal layer was isolated, and a semiquantitative reverse transcription polymerase chain reaction was performed. Immunohistochemical study revealed VEGF in the oviduct luminal epithelium, smooth muscle cells, and blood vessels within the oviduct. VEGF mRNA expression in oviduct was the highest during the periovulatory stage, and the expression in the ampullary and infundibular regions was higher than that in the isthmus. There was a significant positive correlation between serum FSH and LH concentrations and VEGF mRNA expression. There was no significant correlation between serum estradiol and progesterone concentrations and VEGF mRNA expression. These results suggest that VEGF in human oviduct may play an important role related the early reproductive events, which occur predominantly in the ampulla during the periovulatory phase when serum FSH and LH concentrations are high.


http://www.biolreprod.org/cgi/content/abstract/69/4/1201

Testicular secretion of estradiol is necessary for normal spermatogenesis and male reproductive physiology in humans and rodents. The role of estradiol in nonmammalian vertebrates remains unknown, but elevated circulating estradiol has been reported in male lizards, alligators, and various bird species. We have been unable to detect circulating estradiol in male alligators; therefore, we reexamined the question of testicular production of estradiol in alligators using more rigorous assay procedures. A large pool of plasma from a male alligator was extracted and run through an HPLC column. Immunoreactive estradiol-like material eluted coincident with authentic estradiol. By using an ultrasensitive RIA and processing large volumes of male plasma (1000 {micro}l), we were able to measure estradiol. Estradiol in male alligators ranged from 0.23 to 3.14 pg/ml, whereas estradiol in immature female alligators ranged from 14 to 66 pg/ml. Aromatase activity in microsomes from adult alligator ovarian tissue was 36.2 {+/-} 1.6 pmol mg-1 h-1, whereas activity in testicular microsomes ranged between 0.92 and 2.38 pmol mg-1 h-1. Ovarian aromatase activity was inhibited in a concentration-dependent fashion by Fadrozole, but the essentially background activity of testicular aromatase was not inhibited at any concentration of Fadrozole. Likewise, a comparison of alligator testicular and ovarian aromatase mRNA expression gave a similar result: the ovarian expression was 600-fold higher and brain tissue was 10-fold higher than that of the testis. Circulating estradiol in male alligators is probably of
extragonadal origin, and the testis produces little if any of this steroid.


http://www.biolreprod.org/cgi/content/abstract/71/5/1730

A reproducible two-step culture system for isolated mouse ovarian follicles smaller than 100 {micro}m (type 3a follicles) was designed. First, isolated follicles were grown in single droplets of {alpha}-minimal essential medium (MEM) without (deoxy)ribonucleosides at a lower concentration of fetal bovine serum (FBS; 1%) for 6 days with mechanical prohibition of thecal cell attachment. Growing follicles reaching at least 100 {micro}m were transferred to {alpha}-MEM medium enriched with a higher concentration (5%) of FBS to allow attachment and were cultured subsequently for an additional 12 days. Overall, more than 85% of the follicles survived the first culture step, and oocyte growth and granulosa cell proliferation had increased by 25% (P < 0.05). Follicle survival at Day 18 was related to initial follicle diameters at isolation. Average meiotic maturation rates and estrogen secretion were lower compared to those of cultures starting with early preantral follicles of 100-130 {micro}m. Although reverse transcription-polymerase chain reaction analysis revealed the presence of LH-receptor mRNA in thecal cells, an exogenous androstenedione replacement resulted in an increase of estrogen production, suggesting substrate insufficiency. The time needed to grow from early preantral stages to in vitro ovulation is strongly dependent on the initial follicle diameter at isolation. Morphological characteristics of cultured follicles were suggestive for combined transforming growth factor (beta) deficiencies during in vitro culture.


http://www.biolreprod.org/cgi/content/abstract/69/1/106

Platelet-activating factor (PAF) is an autocrine trophic/survival factor for the preimplantation embryo. PAF induced an increase in intracellular calcium concentration ([Ca2+]i) in the 2-cell embryo that had an absolute requirement for external calcium. L-type calcium channel blockers (diltiazem, verapamil, and nimodipine) significantly inhibited PAF-induced Ca2+ transients, but inhibitors of P/Q type ([omega]-agatoxin; [omega]-conotoxin MVIIC), N-type ([omega]-conotoxin GVIA), T-type (pimozide), and store-operated channels (SKF 96365 and econazole) did not block the transient. mRNA and protein for the [alpha]1-C subunit of L-type channels was expressed in the 2-cell embryo. The L-type calcium channel agonist ([+/-]-) BAY K 8644 induced [Ca2+]i transients and, PAF and BAY K 8644 each caused mutual heterologous desensitization of each other's responses. Depolarization of the embryo (75 mM KCl) induced a [Ca2+]i transient that was inhibited by diltiazem and verapamil. Whole-cell patch-clamp measurements detected a voltage-gated channel (blocked by diltiazem, verapamil, and nifedipine) that was desensitized by prior responses of embryos to exogenous or embryo-derived PAF. Replacement of media Ca2+ with Mn2+ allowed Mn2+ influx to be observed directly; activation of a diltiazem-sensitive influx channel was an early response to PAF. The activation of a voltage-gated L-type calcium channel in the 2-cell embryo is required for normal signal transduction to an embryonic trophic factor.

We have developed a method to monitor noninvasively, quantitatively, and in real-time transcription in living preimplantation mouse embryos by measuring expression of a short half-life form of enhanced green fluorescent protein (EGFP) following microinjection of a plasmid-borne EGFP reporter gene. A standard curve was established by injecting known amounts of recombinant green fluorescent protein, and transcriptional activity was then determined by interpolating the amount of fluorescence in the DNA-injected embryos. This approach permitted multiple measurements in single embryos with no significant detrimental effect on embryonic development as long as light exposure was brief (<30 sec) and no more than two measurements were made each day. This method should facilitate analysis of the regulation of gene expression in preimplantation embryos; in particular, during the maternal-to-zygotic transition, and in other species in which limited numbers of embryos are available.


In cattle, administration of retinol at the time of superovulation has been indirectly associated with enhanced developmental potential of the embryo. Vitamin A and its metabolites influence several developmental processes by interacting with 2 different types of nuclear receptors, retinoic acid receptors and retinoid X receptors (RXRs). Given the limited information available concerning the RXR-mediated retinoid signaling system, particularly in species other than rodents, this study was performed to gain insight into the potential role of retinoid signaling during preattachment embryo development in the cow. Bovine embryos were produced in vitro from oocytes harvested from abattoir ovaries and frozen in liquid nitrogen at the oocyte, 2-, 4-, 8-, and 16- to 20-cell, morula, blastocyst, and hatched blastocyst stages. Reverse transcription polymerase chain reaction (PCR) and whole mount in situ hybridization were utilized to investigate mRNA expression for RXR(alpha), RXR(beta), RXR(gamma), alcohol dehydrogenase I (ADH-I), retinaldehyde dehydrogenase 2 (RALDH2), peroxisome proliferator activated receptor gamma (PPAR(gamma)), and glyceraldehyde-3-phosphate dehydrogenase. Transcripts for RXR(alpha), RXR(beta), RALDH2, and PPAR(gamma) were detected in all stages beginning from the oocyte through to the hatched blastocyst. Whole mount in situ hybridization performed using digoxigenin-labeled antisense probes detected all 4 transcripts in both the inner cell mass and the trophectoderm of hatched blastocysts. PCR products obtained for ADH-I exhibited very low homology to known human and mouse sequences. Immunohistochemistry was performed using polyclonal anti-rabbit antibodies against RXR(beta) and PPAR(gamma) to investigate whether these embryonic mRNAs were translated to the mature protein. Strong immunostaining was observed for both RXR(beta) and PPAR(gamma) in the trophectoderm and inner cell mass cells of intact and hatched blastocysts. Messenger RNA was not detected at any stage for RXR(gamma).

Expression of mRNA for RXR(alpha), RXR(beta), RALDH2, and PPAR(gamma) suggests that the early embryo may be competent to synthesize retinoic acid and regulate gene expression during preattachment development in vitro.

The production of animals with large transgenes is an increasingly valuable tool in biotechnology and for genetic studies, including the characterization and manipulation of large genes and polygenic traits. In the present study, we describe an intracytoplasmic sperm injection (ICSI) method for the stable incorporation and phenotypic expression of large yeast artificial chromosomes (YAC) constructs of submegabase and megabase magnitude. By coinjecting spermatozoa and YACs into metaphase II oocytes, we were able to produce founders exhibiting germine transmission of an intact and functional transgene of 250 kilobases, carrying the mouse tyrosinase locus, used here as a reporter gene to rescue the albinism of recipient mice. More than 35% transgenesis was obtained for this YAC transgene. When compared with the pronuclear microinjection standard method, the efficiency of the ICSI-mediated YAC transfer system was significantly greater. In summary, we describe, for the first time, stable incorporation in the host genome and correct phenotypic expression of large DNA constructs mediated by ICSI.


Proper distribution of immune cells in the uterus is a prerequisite for successful implantation and subsequent placentation, but biochemical signals that govern such events have not been well characterized. In the present study, the cDNA of a chemokine, interferon (IFN)-(gamma)-inducible protein 10 kDa (IP-10), was identified from a cDNA subtraction study between uterine endometrial tissues from Day 17 pregnant and Day 15 cyclic ewes. The effect of IFN-(alpha), IFN-(gamma), and IFN-(tau) on IP-10 expression and the involvement of IP-10 in the recruitment of immune cells were then investigated. Northern blot analysis revealed that large amounts of IP-10 mRNA were present during conceptus attachment to maternal endometrium and early placentation. IP-10 mRNA was localized to monocytes distributed in the subepithelial stroma of pregnant but not cyclic uteri. This finding was supported by the discovery of IP-10 mRNA expression in monocytes but not in lymphocytes, uterine epithelial cells, or stromal cells. Moreover, the expression of IP-10 mRNA by the monocytes was stimulated by IFN-(alpha), IFN-(gamma), and IFN-(tau) in a dose-dependent manner, but the expression of IP-10 mRNA by the endometrial explants was most stimulated by IFN-(tau). In a chemotaxis assay, migration of peripheral blood mononuclear cells was stimulated by the addition of IFN-(tau) stimulated-endometrial culture medium, and the effect was significantly reduced by neutralization with an anti-IP-10 antibody. These results suggest that endometrial IP-10 regulated by conceptus IFN-(tau) regulates recruitment and/or distribution of immune cells seen in the early pregnant uterus.


Molecular determinants and mechanisms involved in ovarian follicular growth, ovulation and luteinization are not well understood. The objective of this study was to identify genes expressed in bovine granulosa cells (GC) of dominant follicles (DF) and down-regulated after hCG-induced ovulation, using the suppression subtractive hybridization (SSH). GC were collected from DF at day 5 of the estrous cycle and from ovulatory follicles (OF) obtained 23 h following injection of...
hCG. A subtracted cDNA library (DF-OF) was generated and screened using unsubtracted (DF, OF) and subtracted (DF-OF, OF-DF) cDNAs as complex 32P-probes. A total of 32 non-redundant cDNAs were identified: 23 cDNAs matched with sequences of known biological function and 9 cDNAs with complete or partial sequences of undefined biological function. Detection of genes known to be down-regulated during the periovulatory period in the bovine species, such as CPD, CYP11A1, CYP19A1, FSHR, LRP8/ApoER2 and SERPINE2, validated the physiological model and analytical techniques used. For a subset of genes such as ARFGAP3, CYP11A1, CYP19A1, FSHR, FST, GJA1, IDH3, INHBA, LHCGR, LHCGR lacking exon 10, PRC1, PRG1, RPA2, SCD and TRIB2, gene expression profiles were compared by virtual Northern blot or reverse transcriptase-polymerase chain reaction from follicles obtained at different developmental stages. Results confirmed a down-regulation of the respective mRNAs in GC of OF compared with that of DF. We conclude that we have identified novel genes that are down-regulated by hCG in bovine GC of DF during the periovulatory period, which may contribute to follicular growth, ovulation and/or luteinization.


http://www.biolreprod.org/cgi/content/abstract/68/6/1997

The ovarian steroid hormones, estrogen and progesterone, have important roles in establishing the new vascular bed within the endometrium during each menstrual cycle; however, little is known about the mechanisms underlying this process. We recently showed that mRNA and protein levels for the angiogenic factor vascular endothelial growth/permeability factor (VEG/PF) in endometrial glandular epithelial and stromal cells of baboons were decreased to very low levels by ovariectomy, and we proposed that the levels of estrogen and progesterone exhibited during the menstrual cycle regulate endometrial VEG/PF expression in the primate. To test this hypothesis, VEG/PF mRNA levels were determined by reverse transcription-polymerase chain reaction in glandular epithelial and stromal cells isolated by laser-capture microdissection from, and VEG/PF protein was determined by immunocytochemistry in the endometrium of baboons after ovariectomy and chronic administration of estradiol and progesterone in levels designed to replicate the hormonal profiles that are characteristic of the proliferative and secretory phases of the menstrual cycle. Administration of estradiol to ovariectomized baboons in levels that replicated the late-proliferative phase of the menstrual cycle (209 {+/-} 40 pg/ml serum) increased/restored VEG/PF mRNA to levels in the glands (5.57 {+/-} 1.53 amol/fmol 18S rRNA, P < 0.01) and stroma (2.61 {+/-} 1.57 amol/fmol 18S rRNA, P < 0.02) that were approximately 10-fold greater than those observed after ovariectomy alone (0.52 {+/-} 0.21 and 0.22 {+/-} 0.11 amol/fmol 18S rRNA, respectively) and were similar to those previously shown in intact baboons. Concomitant administration of estradiol and progesterone to ovariectomized baboons in levels that replicated the midsecretory phase of the menstrual cycle (44 {+/-} 15 pg/ml serum and 9.8 {+/-} 2.2 ng/ml serum, respectively) resulted in glandular epithelial (3.65 {+/-} 1.42 amol/fmol 18S rRNA) and stromal (1.25 {+/-} 0.77 amol/fmol 18S rRNA) VEG/PF mRNA levels that were not significantly different from those exhibited after ovariectomy or ovariectomy and estradiol treatment. Comparable results were obtained for VEG/PF mRNA expression in whole-endometrial tissue, although the relative 2-fold increase (P < 0.03) in VEG/PF mRNA levels induced by estrogen in mixed endometrial cells of ovariectomized baboons appeared to be less marked than that in isolated glandular epithelial and stromal cells. After ovariectomy, endometrial width (0.98 {+/-} 0.09 mm) was approximately one-third of that in intact baboons (3.58 {+/-} 0.32 mm), and endometrial VEG/PF protein expression was low. Estradiol restored endometrial width (3.00 {+/-} 0.12 mm, P < 0.01) and VEG/PF protein expression to normal. In summary, estrogen has a significant role in regulating and maintaining VEG/PF expression by glandular epithelial and stromal cells of the endometrium during the menstrual cycle.

http://www.biolreprod.org/cgi/content/abstract/69/4/1265

The growth and development of follicles within the ovary are highly dependent on autocrine and paracrine signaling involving growth factors from granulosa cells, theca cells, stromal interstitial cells, and the oocytes. The growth factor bone morphogenetic protein-4 (BMP-4) and its receptor (BMPR-IB) have been detected in ovaries, and a mutation in BMPR-IB has been associated with abnormal ovulation rate. The objective of the current study was to examine the role that BMP-4 plays in the early stages of primordial follicle development. Ovaries from 4-day-old rats were placed into a whole-ovary organ culture system for 2 wk to investigate the effect that treatment with exogenous BMP-4 has on early follicle development. BMP-4-treated ovaries had a significantly higher proportion of developing primary follicles and fewer arrested primordial follicles than did untreated controls. This indicates that BMP-4 promotes primordial follicle development and the primordial-to-primary follicle transition. Ovaries were also treated with neutralizing antibody against BMP-4 to determine effects of removing endogenously produced BMP-4. Interestingly, ovaries treated with BMP-4 antibody were markedly smaller than controls. This was associated with a progressive loss of oocytes and primordial follicles, a progressive increase in cellular apoptosis, and an accompanying loss of normal ovarian tissue morphology over time. Immunocytochemistry localized BMP-4 protein to isolated stromal cell populations, selected stromal cells (i.e., pretheca cells) associated with developing primordial follicles, and the basement membrane of follicles. Ovaries were treated with BMP-4 and RNA collected after organ culture to determine whether BMP-4 signaling affects expression of other growth factors. Kit ligand and basic fibroblast growth factor expression was unchanged, but TGF(alpha) expression was decreased in whole ovaries. Taken together, these data suggest that BMP-4 plays an important role in promoting the survival and development of primordial follicles in the neonatal ovary.


http://www.biolreprod.org/cgi/content/abstract/67/1/147

REP38 is a rabbit epididymal secretory protein of 38 kDa that has recently been shown to interact with spermatozoa. A rabbit epididymal cDNA expression library was screened with a polyclonal antibody raised against REP38. A single clone (REP38-c1) with an open reading frame encoding a polypeptide of 666 amino acids was obtained. Cleavage of a 22-amino acid N-terminal signal peptide revealed a mature protein with a theoretical molecular mass of 74.5 kDa. Northern blot analysis revealed the presence of two cross-hybridizing transcripts of approximately 1.3 and 2.5 kilobases that appear to result from alternative mRNA splicing. This finding may explain the discrepancies between the observed (38 kDa) and deduced molecular mass of REP38. Expression of both transcripts was epididymis specific and was detected only in regions 2-6. During development, the expression of REP38-c1 mRNA was initiated between 1 and 2 mo postnatum and therefore precedes the appearance of sperm within the lumen of the epididymis. These findings are in agreement with the immunohistochemical localization of the REP38 protein. Androgen deprivation induced by orchidectomy reduced REP38-c1 mRNA levels below the limit of detection, an effect that was reversed by administration of exogenous testosterone. Although REP38-c1 mRNA was detected only in the rabbit epididymis, database searches indicated homology with two rat testis specific cDNAs, KTT4 and odf2, which encode sperm outer dense fiber proteins.

http://www.biolreprod.org/cgi/content/abstract/69/3/1042

This study addresses the role of cAMP hydrolytic isoenzyme phosphodiesterase type 3 (PDE 3) modulation on human oocyte maturation in vitro. Presence of phosphodiesterase type 3 A (PDE 3A) mRNA was confirmed in human germinal vesicle-stage (GV) oocytes. Making use of a selective PDE 3 inhibitor, Org 9935 (10 {micro}M), oocytes retrieved from immature follicles were arrested in prophase I with a high efficiency for up to 72 h. Cumulus oocyte complexes (COCs) were retrieved in the follicular phase of the cycle before or after exposure to endogenous LH or hCG administration in vivo and randomly distributed into maturation medium with or without the PDE 3 inhibitor. Previous exposure of small follicles to LH activity in vivo had no influence on the arresting capacity of the PDE 3 inhibitor. Reversal from pharmacological arrest leads to a progression through meiosis in a normal time frame with formation of a well-aligned metaphase plate. Ultrastructure analysis of COC derived from follicles between 8 and 12 mm showed that the induced extension of prophase I arrest in vitro resulted in cytoplasm changes but not in apparent nuclear changes during culture.


http://www.biolreprod.org/cgi/content/abstract/70/1/191

Tumor necrosis factor-{alpha} (TNF{alpha}) has been shown to be a potent stimulator of prostaglandin (PG) F2{alpha} synthesis in bovine endometrial stromal cells. The aims of the present study were to determine the effect of interferon-{tau} (IFN{tau}) on TNF{alpha}-stimulated PGF2{alpha} synthesis and the intracellular mechanisms of TNF{alpha} and IFN{tau} action in the stromal cells. When cultured bovine stromal cells were exposed to TNF{alpha} (0.006-0.6 nM) for 24 h, the production of PGF2{alpha} and cyclooxygenase (COX)-2 gene expression were stimulated by TNF{alpha} (0.06-0.6 nM, P < 0.05). Moreover, a specific COX-2 inhibitor (NS-398; 5 nM) blocked the stimulatory effect of TNF{alpha} on PGF2{alpha} production (P < 0.05). Although IFN{tau} (0.03-30 ng/ml) did not stimulate basal PGF2{alpha} production in the stromal cells, it suppressed TNF{alpha} action in PGF2{alpha} production dose dependently (P < 0.05). Moreover, the stimulatory effect of TNF{alpha} (0.6 nM) on COX-2 gene expression was completely blocked by IFN{tau} (30 ng/ml; P < 0.05), although the gene expression of COX-2 was not influenced by IFN{tau}. The overall results indicate that the stimulatory effect of TNF{alpha} on PGF2{alpha} production is mediated by the up-regulation of COX-2 gene expression and suggest that one of the mechanisms of the inhibitory effect of IFN{tau} on luteolysis is the inhibition of TNF{alpha} action in PGF2{alpha} production in the stromal cells by the down-regulation of COX-2 gene expression stimulated by TNF{alpha}.


http://www.biolreprod.org/cgi/content/abstract/68/1/45

The purpose of this study was to determine if the nutrition of the oocyte donor ewe influenced the
success of somatic cell cloning. Merino ewes were fed at either a high- or a low-nutrition level for 3-5 mo before superovulation treatments. Freshly ovulated oocytes were enucleated and fused with serum-starved adult granulosa cells, and resulting reconstructed embryos were cultured for 6 days in modified synthetic oviduct fluid. Embryo cleavage and development to blastocysts were recorded, and good-quality embryos were transferred to synchronized recipient ewes either fresh or, on a few occasions, after vitrification. Pregnancies were monitored by ultrasonography from Day 40 of pregnancy, and offspring were delivered by either cesarean section or vaginal delivery. No differences occurred in the numbers of follicles aspirated, oocytes recovered, or of oocytes utilizable for cloning between the high and low groups. Neither were there treatment differences in development to the blastocyst stage. However, transfer of embryos from the high group led to significantly more pregnancies and implanted fetuses. Also, more of the established pregnancies from the high group were carried to term, although this difference was not statistically significant. Lamb mortality was high, with half the live-born perishing soon after birth and more succumbing to various infections within days or weeks of birth, but no clear association between the offspring fate and the treatment group could be established. These results suggest that more research into the effect of nutrition on oocyte quality and its subsequent effect on cloning is warranted.


http://www.biolreprod.org/cgi/content/abstract/72/3/546

Using an interwoven-loop experimental design in conjunction with highly conservative linear mixed model methodology using estimated variance components, 18 genes differentially expressed between nuclear transfer (NT)- and in vitro fertilization (IVF)-produced embryos were identified. The set is comprised of three intermediate-filament protein genes (cytokeratin 8, cytokeratin 19, and vimentin), three metabolic genes (phosphoribosyl pyrophosphate synthetase 1, mitochondrial acetoacetyl-coenzyme A thiolase, and (alpha)-glucosidase), two lysosomal-related genes (prosaposin and lysosomal-associated membrane protein 2), and a gene associated with stress responses (heat shock protein 27) along with major histocompatibility complex class I, nidogen 2, a putative transport protein, heterogeneous nuclear ribonuclear protein K, mitochondrial 16S rRNA, and ES1 (a zebrafish orthologue of unknown function). The three remaining genes are novel. To our knowledge, this is the first report comparing individual embryos produced by NT and IVF using cDNA microarray technology for any species, and it uses a rigorous experimental design that emphasizes statistical significance to identify differentially expressed genes between NT and IVF embryos in cattle.


http://www.biolreprod.org/cgi/content/abstract/71/6/1862

Appropriate growth, development, and function of the placenta is central to the success of nutrient partitioning between the mother, placenta, and fetus. Hormones such as placental lactogen (PL) and leptin are produced in the bovine placenta and play an important role in nutrient partitioning and regulation of placental and fetal growth. Nuclear transfer pregnancies are associated with a number of fetal and placental abnormalities, including increased placental growth and macrosomia, and hence represent a unique situation to gain insight into fetoplacental growth regulation. We have examined the expression of bovine PL (bPL) and leptin in placentomes of artificially inseminated (AI), in vitro produced (IVP), and nuclear transfer (NT) pregnancies at Days 50, 100, and 150 of gestation in the cow. Immunolocalization studies
showed that spatial and temporal patterns of expression of bPL and leptin were markedly altered in the placentomes of NT pregnancies compared with AI or IVP controls. Concentrations of bPL in allantoic fluid, as determined by radioimmunoassay (RIA), were significantly higher (P ≤ 0.001) in NT pregnancies (17.9 ± 3.2 ng/ml; mean ± SD) compared with AI (2.03 ± 1.5 ng/ml), but not IVP (23.4 ± 12.8 ng/ml) pregnancies on Day 150 of gestation. In contrast, amniotic fluid levels of bPL were significantly decreased in NT pregnancies at Day 150 gestation. Leptin mRNA expression, as determined by real-time reverse transcription-PCR, was increased 2.4- to 3.0-fold in NT placentomes compared with AI controls at all gestational ages examined. We speculate that the observed dysregulation of expression of bPL and leptin in NT placentomes could contribute to aberrations in cell migration and invasion and subsequently to alterations in placental metabolism and transfer of nutrients to the fetus, thus leading to increased placental and fetal macrosomia in NT pregnancies.


http://www.biolreprod.org/cgi/content/abstract/71/5/1694

Vascular endothelial growth/permeability factor (VEG/PF) has an established role in angiogenesis, however, the regulation of placental VEG/PF expression during primate pregnancy is incompletely understood. A temporal study was conducted in baboons to determine the effect of acute administration of estradiol on the expression of VEG/PF by cells of the villous placenta. VEG/PF mRNA levels were determined by reverse transcription-polymerase chain reaction in isolated placental cell fractions of baboons after acute i.v. and i.m. administration of estradiol. Within 2 h of estradiol treatment, VEG/PF mRNA (attomoles/ micrograms total RNA) increased within villous cytotrophoblasts to a level (mean ± SEM, 12 612 ± 2419) that was almost 2-fold greater (P < 0.05) than in untreated controls (6810 ± 1368). Cytotrophoblast VEG/PF mRNA levels remained elevated (P < 0.01) 6 h after estradiol treatment (15 006 ± 506), but were not different from controls 18 h after estradiol administration. VEG/ PF mRNA levels in whole villous tissue also were greater 6 h (12 667 ± 2284, P < 0.05) and 18 h (16 080 ± 3816, P < 0.01) after estradiol treatment than in untreated animals (3380 ± 594). In contrast, VEG/PF mRNA levels in cells of the inner villous core were not altered by estradiol treatment. Expression of both the VEG/PF121 and VEG/PF165 mRNA species appeared to increase in the placenta 6 h after estradiol treatment of baboons. We propose that estrogen regulates VEG/PF expression within the placenta in a cell-specific manner, providing a paracrine system to promote vascularization of the villous placenta during the first half of primate pregnancy.


http://www.biolreprod.org/cgi/content/abstract/66/1/190

Ovarian theca cells are the predominant source of gonadotropin-stimulated androgen biosynthesis in vivo. Troglitazone (TG), a synthetic agonist of the peroxisome proliferator-activated receptor (gamma) (PPAR(gamma)) and a thiazolidinedione used to treat insulin resistance, decreases serum androgen concentrations in women with hyperthecosis and/or polycystic ovary syndrome. Using reverse transcription-polymerase chain reaction (RT-PCR), we demonstrated the presence of PPAR(gamma) mRNA in the porcine ovary. Since activation of ovarian PPAR(gamma) may alter hormone-stimulated steroidogenesis in vitro, we cultured porcine theca cells for 48 h in the presence of two different PPAR(gamma) ligands, TG and 15-deoxy-(Delta)12,14-prostaglandin J2 (15d-PGJ2). Putative TG-mediated activation of
PPAR(\(\gamma\)) resulted in a 53%-69% decrease in LH- and/or insulin-stimulated androstenedione and testosterone accumulation. Although TG reduced 3-isobutylmethylxanthine-enhanced LH-stimulated cAMP accumulation by 74%-78%, it did not alter basal cAMP concentrations. Exposure to 8Br-cAMP did not overcome the TG-induced inhibition of androgen accumulation. In contrast, TG administration amplified basal and hormone-stimulated progesterone accumulation, particularly in the presence of insulin, without altering levels of 17(\(\alpha\))-hydroxyprogesterone. The putative natural PPAR(\(\gamma\)) ligand, 15d-PGJ2, inhibited androgen biosynthesis and stimulated progesterone production. RT-PCR-based amplification of cytochrome P450 cholesterol side-chain cleavage (CYP11A) and cytochrome P450 17(\(\alpha\))-hydroxylase/C-17,20-lyase (CYP17) transcripts indicated that TG moderately enhanced expression of these genes. However, TG did not affect CYP17 protein expression. We conclude that putative ligand-mediated activation of PPAR(\(\gamma\)) decreases LH- and/or insulin-driven theca cell androgen production by impairing the ability of CYP17 to synthesize androstenedione from available progestins. The corresponding augmentation of progesterone production could suggest that PPAR(\(\gamma\)) activation induces theca cell differentiation toward a progestin-synthesizing phenotype.


http://www.biolreprod.org/cgi/content/abstract/66/4/976

The bovine placenta produces estrogens from the first trimester until the end of its life span. However, with the exception of the immediate prepartal and intrapartal phases, in which an involvement of placental estrogens has been suggested for the preparation of parturition, their function has not been elucidated yet. To test for a role of placental estrogens as local factors regulating placental growth and differentiation, placentomes from cows that were pregnant for 150, 220, 240, and 270 days, and parturient cows (3 animals per group) were screened immunohistochemically for the expression of estrogen receptor \((\alpha)\) (ER(\(\alpha\))). Indirect immunoperoxidase staining methods were applied using primary monoclonal antibodies (pmAbs) directed against the C-terminus (AER311, HT277) or the N-terminus (AER314, 1D5) of the ER(\(\alpha\)) molecule. Both types of pmAbs identified ER(\(\alpha\)) in stromal cells and capillary pericytes of the maternal caruncular septae. Using pmAb 1D5, the mean percentage of ER(\(\alpha\))-positive caruncular stromal cells decreased from 39.0% (+/-5.9% in pregnant cows to 17.5% (+/-8.3% at parturition (P = 0.011). Only pmAb recognizing the C-terminus identified ER(\(\alpha\)) in the caruncular epithelium, in which positive reactions were found in all cells, with the exception of areas adjacent to the chorionic plate and to major chorionic villi, where the specific signal gradually faded and occasionally disappeared. No positive reactions were observed in the fetal part of the placentomes. The expression of ER(\(\alpha\)) in bovine placentomes was further confirmed by the detection of ER(\(\alpha\))-specific mRNA by reverse transcriptase-polymerase chain reaction and by Western blot analysis. The results suggest a role for placental estrogens as paracrine factors involved in the regulation of placental growth and differentiation.


http://www.biolreprod.org/cgi/content/abstract/66/2/393

Interferon tau (IFN(\(\tau\))) is the pregnancy recognition signal produced by the conceptus trophectoderm and acts in a paracrine manner on the ovine endometrium to increase expression of IFN-stimulated genes primarily in the stroma and deep glandular epithelium, including IFN regulatory factor-1 (IRF-1). The roles of Stat1, Stat2, and IRF-9 in IFN(\(\tau\)) regulation of IRF-1
expression were determined using human stromal fibroblasts lacking specific IFN signaling components or complemented with specific Stat1 mutants. In parental (2fTGH) cells treated with IFN(\tau), Stat1(\alpha)/(\beta) was tyrosine phosphorylated by 15 min, and IRF-1 mRNA and protein increased from 0 to 6 h, was maximal at 6 h, and decreased to 24 h. In contrast, IFN(\tau) did not affect IRF-1 expression in Stat1- and Stat2-deficient cells or in Stat1-deficient cells complemented with Stat1 Y701Q or Stat1 R602L mutants. In Stat1-deficient cells complemented with the Stat1 S727A mutant, Stat1(\alpha), or Stat1(\beta) and treated with IFN(\tau), IRF-1 increased from 0 to 6 h, was maximal at 6 h, and decreased thereafter. In IRF-9-deficient cells stimulated with IFN(\tau), IRF-1 increased from 0 to 6 h but did not exhibit the sharp decline from 6 to 12 h observed in other cells. Collectively, results indicate that IFN(\tau) effect on IRF-1 expression is primarily regulated by tyrosine-phosphorylated Stat1(\alpha) or Stat1(\beta) dimers, whereas the decline of IRF-1 after 6 h of IFN(\tau) treatment is regulated by IRF-9.


http://www.biolreprod.org/cgi/content/abstract/66/3/754

Fas antigen (Fas) is a cell surface receptor that triggers apoptosis in sensitive cells when bound to the Fas ligand (Fas L). The present study was undertaken to identify the presence of a Fas-Fas L system in bovine corpus luteum (CL) and to evaluate the regulation of Fas-mediated luteal cell death by leukocyte-derived cytokines. The reverse transcription-polymerase chain reaction showed higher levels of Fas mRNA expression in CL in the regressed luteal stage (Days 19-21) than in the other stages (P < 0.05). Bovine luteal cells from midcycle CL (Days 8-12) were exposed for 24 h to interferon (\gamma) (IFN; 50 ng/ml) and/or tumor necrosis factor (alpha) (TNF; 50 ng/ml). After 24 h of culture, the expression of Fas mRNA was detected in the cultured cells and was increased by IFN. Moreover, TNF augmented the stimulatory action of IFN, whereas TNF alone did not affect the expression of Fas mRNA. The effects of IFN and TNF on Fas-mediated cell death were also examined. Cells were exposed to IFN and/or TNF for 24 h and were further treated with IFN and/or TNF in the presence or absence of Fas L (100 ng/ml) for 24 h. Treatments of the cells with IFN alone and in combination with TNF resulted in killing of 30% and 50% of the cells (P < 0.05), respectively, whereas TNF alone did not have a cytotoxic effect on the cells. On the other hand, Fas L killed 80% of the cells treated with IFN (P < 0.01) and 85% of the cells treated with the combination of TNF and IFN (P < 0.01), respectively, whereas Fas L showed no effect on the viability of the luteal cells treated with or without TNF. Furthermore, shrunken nuclei and apoptotic bodies were observed in the cells treated with Fas L in the presence of TNF and IFN. The overall results suggest that a Fas-Fas L system is present in bovine CL and that leukocyte-derived TNF and IFN play important roles in Fas-mediated luteal cell death.


http://www.biolreprod.org/cgi/content/abstract/72/3/643

Both the viability of hen prehierarchal follicles and subsequent differentiation associated with the selection of a single follicle per day into the preovulatory hierarchy depend on circulating FSH and the expression of FSH receptor (FSH-R) in granulosa cells. The present study addresses mechanisms that mediate both basal expression plus selective up-regulation of FSH-R mRNA in granulosa cells from prehierarchal follicles. Results demonstrate that FSH-R mRNA is both expressed and functional in granulosa cells collected from growing prehierarchal follicles as small
as those of 1-2 mm in diameter, as indicated by rapid induction of steroidogenic acute regulatory (StAR) protein expression by FSH in vitro. Real-time polymerase chain reaction determined that relative FSH-R expression within the granulosa layer from individual prehierarchal follicles of 6-8 mm in diameter was similar among the 8-13 follicles within this cohort, with the notable exception that the granulosa layer from a single follicle (presumably the selected follicle) showed elevated expression. Levels of FSH-R mRNA expression were enhanced by both recombinant human (rh) transforming growth factor (TGF) \( \beta \)1 and, to a lesser extent, rh-activin A after 20 h of culture. This stimulatory effect was effectively blocked by mitogen-activated protein (MAP) kinase signaling induced by TGF(\( \alpha \)) treatment. Finally, inhibition of MAP kinase signaling, using the selective inhibitor U0126, promoted FSH-R expression and further enhanced TGF(\( \beta \))1-induced FSH-R expression in vitro. Collectively, results suggest that premature granulosa cell differentiation normally is suppressed by tonic MAP kinase signaling. At the time of follicle selection, a release from inhibitory MAP kinase signaling is proposed to occur, which enables the full potentiation of FSH-R expression mediated by intrafollicular factors.


http://www.biolreprod.org/cgi/content/abstract/70/6/1877

Chemokines and chemokine receptors have been implicated as pivotal players in many physiological and pathological situations, but little is known about the expression and function of chemokines and chemokine receptors at the materno-fetal interface. In this study, we first analyzed the transcription of 18 chemokine receptors in first-trimester human trophoblast cells. Among these receptors, CXCR4 was found highly transcribed. We demonstrated afterward that both CXCR4 and CXCL12 (stromal cell-derived factor-1; SDF-1) were expressed in trophoblast cells. Primary cultured trophoblast cells were also found secreting CXCL12 spontaneously. To identify the functional role of CXCR4/CXCL12 in these cells, we treated trophoblast cells with recombinant human (rh)SDF-1(\( \alpha \)) treatment and analyzed the cell viability and signaling pathway. The results showed that rhSDF-1(\( \alpha \)) increased the viability of trophoblast cells and the activation of extracellular signal-regulated kinases signaling pathway in vitro. Our findings suggest that first-trimester trophoblast cells express functional CXCR4/CXCL12, which may play an important role in early pregnancy such as stimulating trophoblast cell proliferation or differentiation in an autocrine manner.


http://www.biolreprod.org/cgi/content/abstract/70/4/1055

Calcitonin gene-related peptide (CGRP) is a potent vasodilator neuropeptide known to be involved in the regulation of vascular tone. Results of previous studies from our laboratory and others suggest that vascular sensitivity to CGRP is enhanced during pregnancy and that the female sex steroid hormones estradiol-17(\( \beta \)) (E2) and progesterone (P4) may be involved in this process. We hypothesized that CGRP receptors in the mesenteric artery are increased during pregnancy and with sex steroid hormone treatments. In the present study, we investigated whether pregnancy and female sex steroid hormones modulate the CGRP-receptors CGRP-A and CGRP-B in the mesenteric artery in the rat. The CGRP-A receptor consists of calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein 1 (RAMP1); however, the CGRP-B receptor needs to be further characterized. Messenger RNA levels for CRLR and RAMP1 were assessed by reverse transcription-polymerase chain reaction, and CGRP-B
receptor proteins levels were determined by Western blot analysis. In addition, [125I]CGRP binding was measured by Scatchard analysis. Both mRNA for CGRP-A (CRLR and RAMP1) and the protein for CGRP-B receptors in mesenteric arteries were increased with pregnancy compared to nonpregnant, diestrous animals. A P4 antagonist, RU-486, downregulated and P4 upregulated these receptors in mesenteric arteries (P < 0.05) in pregnant rats. In adult ovariectomized rats, P4 upregulated CRLR and RAMP1 mRNA levels as well as [125I]CGRP-binding sites. The CGRP-B-receptor protein levels were significantly (P < 0.05) elevated by P4 and by combined E2 and P4 treatment. Together with earlier findings, these data suggest that increases in the expression of CGRP-A (CRLR and RAMP1) and CGRP-B receptors in mesenteric arteries may be important in reducing vascular resistance and in vascular adaptations that occur during pregnancy; in addition, P4 may be involved in this process.


http://www.biolreprod.org/cgi/content/abstract/68/5/1911

In the adult ovary, pituitary FSH via interaction with its receptor (FSHR) is required for follicular maturation and granulosa cell development. In humans and nonhuman primates, the pool of follicles available for adult ovarian function is established in utero. However, our understanding of the ontogeny and developmental regulation of FSHR in the ovary of the primate fetus is incomplete. Our goal was to determine whether the baboon fetal ovary expresses the full-length FSHR mRNA transcript and whether levels are developmentally regulated. Fetal ovaries were obtained at mid (Day 100) and late (Day 165) gestation (term = Day 184) from untreated baboons and on Day 165 from baboons in which fetal estrogen levels were either decreased by >95% by treatment with the aromatase inhibitor CGS 20267 or restored to 30% of normal by treatment with CGS 20267 plus estradiol benzoate administered s.c. to the mother on Days 100-164. The full-length 2088-base pair FSHR mRNA transcript was expressed in ovaries of adult and fetal baboons untreated or treated with CGS 20267 or CGS 20267 and estrogen. Mean (±SEM) FSHR mRNA levels (ratio of FSHR mRNA:18S rRNA), quantified by reverse transcription polymerase chain reaction, were increased (P < 0.05) 2-fold between mid (0.34 ± 0.06) and late gestation (0.76 ± 0.07), an increase prevented (P < 0.05) in estrogen-depleted baboons (0.44 ± 0.10) and partially restored by treatment with CGS 20267 and estrogen (0.58 ± 0.16). We previously showed that the number of follicles/0.33 mm2 in fetal ovaries of untreated baboons in late gestation was reduced 50% by treatment with CGS 20267 and restored to normal in baboons treated with CGS 20267 and estrogen. Thus, when corrected for the number of follicles/0.33 mm2, FSHR mRNA levels were similar in baboon fetal ovaries untreated (0.010 ± 0.001) or treated with CGS 20267 (0.009 ± 0.002) or CGS 20267 and estrogen (0.007 ± 0.003). We conclude that estrogen plays a major role in regulating ovarian FSHR mRNA expression in the primate fetus, and that the developmental increase in FSHR mRNA levels reflects the estrogen-dependent increase in folliculogenesis (i.e., increased number of granulosa cells and oocytes).


http://www.biolreprod.org/cgi/content/abstract/70/6/1600

Insulin stimulates androgen biosynthesis and the accumulation of CYP17 mRNA and heterogeneous nuclear (hn) RNA in primary cultures of immature swine theca cells. To further assess insulinomimetic transcriptional control, we subcloned 1.007 kilobases (kb) of the 5'-upstream region of the CYP17 gene (-976 to +31 base pairs [bp] to the transcriptional start site)
into a firefly-luciferase reporter construct. Insulin drove transcriptional activity of this probe in a time- and dose-dependent fashion, with maximal stimulation of 2.7- to 3.2-fold after insulin exposure (100 ng/ml) for 6 h. Progressive deletional constructs -839, -473, -174, and -75/+31 bp delineated expected reduction in responsiveness, except paradoxical gain of basal CYP17 promoter activity by the -473/+31-bp sequence. The latter suggests a possible intervening inhibitory sequence. Elimination of all sequences 5'-upstream to -174 bp markedly reduced basal transcriptional activity and abolished insulin action. Point mutation of a presumptive Sp1-like element located within -193/-180 bp inhibited basal and insulin-stimulated luciferase activity of the full-length promoter fragment by 40% and 67%, respectively. Disruption of a contiguous presumptive AP-2 site produced a comparable outcome. Combined mutation of the Sp1 and AP-2-like elements eliminated basal and insulin-potentiated CYP17 promoter activity. By Western analysis, insulin augmented cognate receptor phosphoprotein concentrations by 31-fold within 10 min. Chemical inhibitors of MEK-activated ERK1/2 attenuated insulin-enhanced CYP17 transcriptional activity by 76-80%. In summary, insulin drives transcriptional activity of a 5'-upstream regulatory sequence (-976 to +31 bp) of the swine CYP17 gene in primary cultures of theca cells, under a minimal requirement for combined activity of proximal (-193/180 bp) Sp1 and AP-2-like elements.

Biol. Bull.  (1)


http://www.biolbull.org/cgi/content/abstract/202/1/74

To understand the flexibility of symbiotic associations in coral reefs, we investigated the specificity of the Aiptasia (cf. insignis)-Symbiodinium association in the laboratory by rendering the anemones apsymbiotic and inoculating them with different isolates of Symbiodinium. Infective algal symbionts were monitored over 3 months by re-isolation and identification using denaturing-gradient gel electrophoresis and sequence comparison of their amplified 18S rRNA hypervariable V1 + V2 gene region. Despite similarity in their external morphology, the algal isolates differed in their infectivity towards the host. Within days of single-isolate inoculation, apsymbiotic anemones formed associations with fresh or cultured isolates (clade B) from the anemones Aiptasia sp. or A. tagetes, respectively. They associated to a limited extent with cultured isolates (clade A) from the tridacnids Tridacna crocea or Hippopus hippopus, and not at all with a cultured isolate (clade C) from the stony coral Montipora verrucosa, nor with a free-living isolate (clade A) from subtidal sands. Aposymbiotic anemones inoculated with a mixture of all isolates had only the anemone taxon as their detectable symbionts. Re-inoculation of induced symbioses with a mixture of all isolates and incubation with wild anemones showed that the initial induced symbioses with the anemone taxon were stable. Anemones originally infected with tridacnid isolates either additionally acquired the anemone taxon or had the former outgrown by the latter. These results demonstrate the presence of a host-symbiont recognition mechanism, and possibly competition among potential algal symbionts in the Aiptasia-Symbiodinium association. Here we present a method that may be useful in monitoring the algal population dynamics in symbiotic corals in the field, along with an efficient method of rendering Aiptasia apsymbiotic for further laboratory investigation of Aiptasia-Symbiodinium symbioses.

http://www.sciencedirect.com/science/article/B6V5X-4561BX6-4/2/af3e3ebb8baae6e6e7f59bacf156b778

The arctic fox population in Fennoscandia is on the verge of going extinct after not being able to recover from a severe bottleneck at the end of the 19th century. The Siberian arctic fox population, on the other hand, is large and unthreatened. In order to resolve questions regarding gene flow between, and genetic variation within the populations, a 294 bp long part of the mitochondrial hypervariable region 1 was sequenced. This was done for 17 Swedish, 15 Siberian and two farmed foxes. Twelve variable nucleotide sites were observed, which resulted in 10 different haplotypes. Three haplotypes were found in Sweden and seven haplotypes were found in Siberia. An analysis of molecular variance showed a weak, but significant, differentiation between the populations. No difference in haplotype diversity was found between the populations. A phylogenetic analysis revealed that the three Swedish haplotypes were not monophyletic compared to the Siberian haplotypes. These results indicate a certain amount of gene flow between the two populations, both before and after the bottleneck. Restocking the Fennoscandian population with arctic foxes from Siberia might therefore be a viable option.


http://www.sciencedirect.com/science/article/B6V5X-3TP5RMP-8/2/f8be5e1e93abdd1cbd747b0e64438b8f

Fragmentation of natural habitats is increasing dramatically, yet its effects on the distribution of genetic variation in wild populations remain largely unknown. In this study, two woodland populations of the redback salamander Plethodon cinereus in Connecticut, USA, were contrasted using molecular and morphological markers. One population was from a landscape fragmented for 300 yr by human activities and another from a nearby, undisturbed landscape. Genetic differentiation, based on molecular markers, was marginally greater in the fragmented population than in the contiguous population, and, within the fragmented population, was greater among subpopulations lacking historical forest connections. Genetic divergence between subpopulations was also weakly related to geographic distance in the population occupying continuous forest, but not in the population occupying fragmented forest. Fragmentation enhanced morphotype diversity within populations of P. cinereus, whereas levels of molecular genetic diversity within subpopulations were apparently unaffected.


http://www.sciencedirect.com/science/article/B6V5X-4F7DJSC-4/2/a1857f2122b703e39680bdf3b997b738
In Slovenia, the Adriatic basin inhabited by native marble trout (S. marmoratus), and the Danubian basin inhabited by native Danubian lineage of brown trout (S. trutta) have been intensively affected by stocking with non-native trout strains. In order to assess spread of non-native strains and their introgression with native trout, a population study based on five microsatellite loci was applied across ten marble and ten brown trout populations, ranging from allegedly non-introgressed to heavily managed. On the basis of correspondence analysis, which revealed three clear groupings consisting of the Danubian and Atlantic lineages of brown trout and the marble trout, the alleles, characteristic of each grouping were identified and used for estimating genetic composition of each population according to the three possible origins. Among the wild populations, five marble and one brown trout populations were found to be pure; all the others were introgressed with exotic alleles (Atlantic and marmoratus alleles in the Danubian basin and Atlantic and Danubian in the Adriatic basin) that markedly dominate in intensively managed populations. As revealed by non-significant FIS values, panmixia between native and introduced fishes has for the most part already been reached. Our research showed that it is not only marble trout whose identity is endangered in Slovenia but also the existence of autochthonous Danubian brown trout is critically compromised, which is new information to be taken into account for local trout conservation.


http://www.sciencedirect.com/science/article/B6V5X-44R1B5PB2/e93c432185ada75f583513834ec69971

We examined the effect of selective logging on the genetic diversity of Scaphium macropodum using RAPD markers via two approaches: (1) to investigate the immediate effect by studying a same population before and after logging, and (2) to determine the long term effect by comparing two regenerated stands with an adjacent unlogged stand, assuming that they were genetically identical before logging. Results showed no negative immediate impact for the first approach, probably due to the high abundance and heterogeneity of S. macropodum in the compartment investigated. However, for the latter approach, substantial genetic erosion (i.e. 31.5% reduction for Shannon diversity, H) was detected in one of the regenerated stands corresponding to its extremely low tree density for S. macropodum. This implies the possible occurrence of genetic drift and increased inbreeding due to population decline as a result of logging. However, the observed genetic differences among the three sub-populations having prevailed before logging cannot be totally discounted in the second approach. This study also demonstrates the use of tree density as a good surrogate measure of genetic diversity. The present harvesting system in Malaysia based on a general cutting limit need to be refined; the basis for determining cutting limit in a forest management unit should consider abundance of commercial species.


http://www.sciencedirect.com/science/article/B6WBP-46MJSVB-

http://www.sciencedirect.com/science/article/B6WBP-4F5SB85-1/2/6e0aae81ceb85686c2dc4b7629fe6b34

Pseudomonas syringae pv. tagetis, a plant pathogen being considered as a biological control agent of Canada thistle (Cirsium arvense), produces tagetitoxin, an inhibitor of RNA polymerase which results in chlorosis of developing shoot tissues. Although the bacterium is known to affect several plant species in the Asteraceae and has been reported in several countries, little is known of its genetic diversity. The genetic relatedness of 24 strains of P. syringae pv. tagetis with respect to each other and to other P. syringae and Pseudomonas savastanoi pathovars was examined using 16S-23S rDNA intergenic spacer (ITS) sequence analysis. The size of the 16S-23S rDNA ITS regions ranged from 508 to 548 bp in length for all 17 P. syringae and P. savastanoi pathovars examined. The size of the 16S-23S rDNA ITS regions for all the P. syringae pv. helianthi and all the P. syringae pv. tagetis strains examined were 526 bp in length. Furthermore, the 16S-23S rDNA ITS regions of both P. syringae pv. tagetis and P. syringae pv. helianthi had DNA signatures at specific nucleotides that distinguished them from the 15 other P. syringae and P. savastanoi pathovars examined. These results provide strong evidence that P. syringae pv. helianthi is a nontoxigenic form of P. syringae pv. tagetis. The results also demonstrated that there is little genetic diversity among the known strains of P. syringae pv. tagetis. The genetic differences that do exist were not correlated with differences in host plant, geographical origin, or the ability to produce toxin.


http://www.sciencedirect.com/science/article/B6WBP-49V790D-3/2/4c7e3f5a581f17c2a5b3cb0096acce3

A polymerase chain reaction (PCR) protocol that can be used to distinguish Pseudomonas syringae pv. tagetis from other P. syringae pathovars, including those that induce apical chlorosis in several plants of the Asteraceae family and in pea, and closely related P. savastanoi pathovars was developed based on DNA sequences from P. syringae pv. tagetis that are required for tagetitoxin synthesis. PCR primer sets designated TAGTOX-9 and TAGTOX-10 in PCR amplifications with DNA from most strains of P. syringae pv. tagetis, produced amplicons of 507 and 733 bp, respectively. The same size amplicons were produced in PCR amplifications with bacterial cells isolated from chlorotic leaf tissue from Canada thistle (Cirsium arvense) plants infected with P. syringae pv. tagetis. Among 16 other P. syringae pathovars, only PCR amplifications with DNA from P. syringae pv. helianthi produced the same size amplicons with the respective primer sets. Low levels of the 507-bp amplicon were produced in PCR amplifications with the TAGTOX-9 primers and DNA from P. syringae pv. helianthi or the nontoxigenic strains of P. syringae pv. tagetis. These results suggest that P. syringae pv. helianthi, the most closely related pathovar to P. syringae pv. tagetis, may be a nontoxigenic form of P. syringae pv. tagetis. Results from PCR amplifications with the TAGTOX-9 and TAGTOX-10 primers provide strong evidence that the newly described Pseudomonas syringae pathovars, CT99B016C isolated from Canada thistle and PP105 and Pisum97-1 isolated from pea, which cause apical chlorosis in these respective hosts, are different from P. syringae pv. tagetis.

http://www.sciencedirect.com/science/article/B6WBP-48KVC5M-2/2/5f01759a2fb1d8bd0e2480b1b745fd2c

Salsola tragus L. (Russian thistle, Chenopodiaceae), a weed of Central Asian origin, has two biotypes in California, type A and type B. The gall midge Desertovellum stackelbergi Mamaev (Diptera: Cecidomyiidae), which attacks S. tragus in Uzbekistan, is a candidate biological control agent for this weed in the United States. In a field test conducted in Uzbekistan with plants of the two biotypes of S. tragus from California, both biotypes were attacked by the insect, although type A was the preferred host. Accessions of S. tragus from Uzbekistan, Greece, and Ukraine were similar to the California type A when compared using RAPD and ISSR analyses, while California type B was distinct. Since both California biotypes were hosts to the gall midge, further studies on the biology and host specificity of the insect are justified. Genetic characterization of target weeds can provide information useful for the selection of natural enemies.


http://www.sciencedirect.com/science/article/B6WBP-47MK74W-C/2/03fe1c34a6296d6567f0fe1743eea878

We report on the construction and optimization of recombinant Autographa californica nucleopolyhedrovirus engineered to express the insect-selective toxin IT2 from the scorpion Leiurus quinquestriatius hebraeus. We constructed a series of viruses expressing the synthetic LqhIT2 gene with different signal sequences or controlled by different promoters. The effect of the various viruses on speed of response was assayed in Heliothis virescens larvae. In addition, the performance of the optimum recombinant viral construct was compared with similar constructs carrying the Anuroctonus australis hector insect toxin controlled by two different promoters. There were no significant differences in speed of response of viruses with the early hr5/ie1 the early hr5/lef3, or the early/late hr5/39Kpromoter driving toxin expression. However, the choice of signal sequence resulted in significant effects. The signal sequence from the bombyxin gene of the silkworm Bombyx mori, proved nominally the best. When the signal sequences were used from the following genes, the viruses acted significantly slower: AcMNPV gp67, a lepidopteran adipokinetic hormone, a dipteran chymotrypsin, and the homologous LqhIT2. Finally, the signal sequences of the genes for cuticle protein II of Drosophila melanogaster and of the insect toxins of the scorpions A. australis hector and Hottentota judaicus performed very poorly. The speed of action of AcMNPV, carrying the synthetic LqhIT2 gene with the bombyxin secretion signal and driven by the hr5/ie1 promoter [Ac.LqhIT2(hr5/ie1)], was compared to that of the same virus carrying the AaIT gene under the control of the p10 promoter, or the hr5/ie1 promoter in H. virescens, Trichoplusia ni, and Spodoptera exigua larvae. All recombinant viruses elicited the response significantly faster than the common progenitor wild-type virus in all tests. The response elicited by Ac.LqhIT2(hr5/ie1) was nominally faster than that of both viruses expressing AaIT in all insects tested.

Several isolates of the fungus Phoma macrostoma demonstrated bioherbicidal activity against dandelion seedlings when applied to soil. Weed control ranged from 36 to 100% depending on the isolates and the doses applied. Using microbiological and molecular genetic techniques, the ability of these isolates to colonize target, and nontarget plants and to disperse and persist in soil were determined. PCR primers highly specific to the biocontrol isolates of P. macrostoma, were used to detect the isolates at rates of application between 4 and 1000 g/m². Based on the results from representative isolates tested, it was concluded that P. macrostoma colonized root tissues of both resistant and susceptible crop species and a susceptible weed species grown in treated soil, and the frequency of fungal isolation declined with time. It was occasionally detected on untreated plant tissues, which may have resulted from either natural occurrences on seed, or contamination of soil. The biocontrol fungus appeared to have limited mobility in the soil since it was not often detected away from the area where it was placed. It persisted in the soil at detectable levels for up to 4 months, but then its presence declined with time. One year post application, P. macrostoma was either not present or significantly reduced in both soil and plant samples depending on the year of sampling. The results suggested that the isolates of P. macrostoma used for biological weed control would have minimal environmental impact due to its ubiquitous nature, limited mobility, and weak persistence over seasons.

Biological Psychiatry (12)


BackgroundNeurocognitive deficits are recognized as a cardinal feature of schizophrenia, but the determinants of these deficits remain unknown. Recent reports have suggested that a functional polymorphism, Val158Met in exon III of the catechol-O-methyltransferase gene, shares approximately 4% variance with performance on the Wisconsin Card Sorting Test. These findings led to suggestions that the catechol-O-methyltransferase polymorphism may exert its effects by modulating prefrontal dopamine function, but few other neurocognitive measures have been examined, leaving open questions about phenotypic specificity.MethodsWe examined the effects of the catechol-O-methyltransferase Val158Met polymorphism in 58 individuals with chronic schizophrenia who completed a battery of 15 neurocognitive tests, which were reduced to four reliable neurocognitive domain scores. We examined the effects of genotype on these four domains and on global neurocognitive ability.ResultsThe Met allele was associated with better performance in the Processing Speed and Attention domain, but not with other domain scores measuring executive and visuoperceptual functions, declarative verbal learning and memory, simple motor ability, or global neurocognitive function. Genotype shared approximately 11% of variance with Processing Speed and Attention scores, and approximately 2% of variance with Wisconsin Card Sorting Test scores.ConclusionsThe findings provide independent support for the hypothesis that the catechol-O-methyltransferase Val158Met polymorphism influences neurocognitive function in schizophrenia, and suggest that the functional effects may be expressed on measures of Processing Speed and Attention. This information may prompt reconsideration of the "prefrontal dopamine" hypothesis and invites examination of a broader range of effects in efforts to refine the neurocognitive phenotype that is most relevant to variation
Disturbances in dopaminergic transmission have been implicated in the etiology of psychotic disorders. Interindividual differences in deoxyribonucleic acid (DNA) sequences coding for dopamine receptor proteins might contribute to the genetic background of these diseases. We have identified a variation in exon 1 of the dopamine D4 receptor (DRD4) gene, which is characterized by a polymorphic 12 base pair (bp) repeat. This repeat codes for a sequence of four amino acids in the extracellular N-terminal part of the receptor, which borders the first putative transmembrane domain. The 12bp repeat occurs as a two-fold repeat in the more common variant (A1 allele) and is represented only once in the rarer one (A2 allele). The frequency of this DNA polymorphism was determined in a sample of 59 patients suffering from delusional disorder, in 79 schizophrenic patients, and in 75 control subjects. Sixteen (27%) of the 59 patients with delusional disorder carried the A2 allele compared with six (8%) of the controls. The observed difference in genotype frequencies between patients with delusional disorder and controls was highly significant. There were no significant differences in genotype frequencies between schizophrenics and controls. Our results strongly suggest the involvement of genetic variation in the DRD4 gene in conferring susceptibility to delusional disorder.


Background: Allelic variation at the CYP2D6 gene has been reported to be associated with Parkinsons' disease (PD) and Lewy body dementia (LBD), but not with Alzheimer's disease (AD). AD has been associated with apolipoprotein E (apoE) [epsil]4 allele loading. Methods: We examined CYP2D6 and apoE polymorphisms in a sample of 259 patients with dementia, 210 of whom had a diagnosis of AD, and 107 healthy controls. Results: We found that the allelic frequency in our AD sample did not vary from that in the controls. The debrisoquine hydroxylase poor metabolize phenotype was not more prevalent among AD cases than among controls in contrast to that reported for PD and LBD. We also found that CYP2D6 status does not modify the risk effect for AD conferred by apoE [epsil]4 alleles. Conclusions: These findings provide some support to the notion that, at a genetic level, at least at this locus, AD could be distinct from PD and LBD.
Background

Synapsin II encodes a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles in the presynaptic nerve terminal. The expressions of messenger ribonucleic acid and protein of synapsin II have been reported to be significantly reduced in the brains of schizophrenia patients. The synapsin II gene is located on 3p25, a region that has been implicated to be associated with schizophrenia by genetic linkage. All these findings suggest synapsin II as a candidate gene for schizophrenia.

Methods

In this work, we studied four markers (two single nucleotide polymorphisms (SNPs): rs308963 and rs795009; and two insertion/deletion polymorphisms: rs2307981 and rs2308169) covering 144.2 kilobase pairs (kb) with an average interval of 38 kb in synapsin II in a sample of 654 schizophrenic patients and 628 normal control subjects to explore the mechanism underlying schizophrenia.

Results

We found significant differences in allele frequency distribution of SNP rs795009 (p = .000018, odds ratio 1.405, 95% confidence interval 1.202-1.641) between patients and control subjects. The T allele was significantly higher in patients than in control subjects. Moreover, the overall frequency of haplotype showed significant differences between patients and control subjects (p = .0015).

Conclusions

This study suggests a positive association between synapsin II and schizophrenia, implying that synapsin II is involved in the etiology of schizophrenia.


http://www.sciencedirect.com/science/article/B6T4S-3WJ6WN8-N/2/a50e6d353dc1b2aa8da9c38510b500f

Background: As part of an ongoing, larger study, "Phenomenology and Course of Pediatric Bipolarity", a subset of prepubertal and early adolescent onset bipolar (PEA-BP) probands, on whom trio blood collection was complete, were used to study genetic transmission of the serotonin transporter linked promoter region (HTTLPR) short and long alleles using the transmission disequilibrium test (TDT). The HTTLPR alleles were selected based on postulated serotonergic mechanisms for PEA-BP and on the burgeoning number of HTTLPR allele studies in bipolar (BP) adults.

Methods: There were 46 complete trios of PEA-BP probands and both biological parents. Probands had a mean age of 11.1 +/- 3.0 years and a mean age of onset of PEA-BP of 8.1 +/- 4.0 years. Comprehensive diagnostic assessments included a semi-structured research interview, the WASH-U-KSADS, administered separately to mothers and to children by blind raters. Probands manifested severe impairment (CGAS 43.9 +/- 8.9), elated mood (84.8%), grandiosity (78.3%), rapid cycling (78.3%) and psychosis (63.0%). The HTTLPR length variant was genotyped using fluorescently labeled primers and automated capillary electrophoresis using laser-induced fluorescence.

Results: The TDT was not significant (TDT chi square = .020, df = 1, p = .89).

Conclusions: This negative result is consistent with the one negative TDT and two negative linkage studies of HTTLPR alleles in bipolar adults in the literature.


http://www.sciencedirect.com/science/article/B6T4S-3W4XK23-C/2/54785e1540e50c5a3e61bb81301a64d7

Background: The goal of this study was to evaluate the role of genetic variation in the coding sequence of tryptophan hydroxylase (TPH) in the pathogenesis of several psychiatric diseases in which altered serotonin function has been implicated: bipolar affective disorder (BP), obsessive-compulsive disorder (OCD), anorexia nervosa (AN), seasonal affective disorder (SAD), panic disorder (PD), and alcoholism (Alc).

Methods: Ninety-three percent of the TPH coding sequence
was screened by polymerase chain reaction single-strand conformation polymorphism (SSCP) for DNA sequence variations in 128 AN, 88 OCD, 72 SAD, 45 PD, and 36 BP patients and 142 normal volunteers. Also included in the screening were 61 Alc randomly selected from a Finnish alcoholic population in which an association of a TPH intron 7 polymorphism with suicidality was previously observed. Polymorphisms detected by SSCP were characterized by DNA sequencing and by allele-specific restriction enzyme digestion. Genotyping was then performed in 34 Finnish alcoholic suicide attempters. Results: A rare silent mutation was identified in exon 10 and is designated T1095C. The C1095 allele was found in 1 OCD and in 2 AN subjects; all 3 individuals were heterozygous (C1095/T1095) for the variant allele. No association was observed between this TPH T1095C variant with either OCD, AN, Alc, or suicidality. Conclusion: These results suggest that the coding sequence of the TPH gene does not contain abundant variants, and may not play a major role in vulnerability to several psychopathologies in which reduced serotonin turnover has been implicated.


http://www.sciencedirect.com/science/article/B6T4S-40NMS5D-7/2/cb3246537bc2f2dc3dd0b9f4603806da

Background: Substantial evidence indicates that lithium may exert its therapeutic effects through progressive adaptive changes at the level of gene expression; however, the study of lithium-regulated genes has been primarily undertaken with the "candidate gene" approach based on a specific testable hypothesis. The aim of our study was to identify lithium-regulated genes that would not be predicted a priori by the candidate gene approach. Methods: Differential display polymerase chain reaction was used to isolate and identify messenger RNAs (mRNAs) that are differentially expressed in the frontal cortex of rats given lithium for 5 weeks to achieve plasma lithium concentrations of 0.6 to 0.9 mmol/L. Results: A putative lithium-regulated complementary DNA fragment (LRG1) was identified. Northern blot analysis revealed that 5 weeks of lithium treatment, but not 1 week, significantly reduced LRG1 mRNA levels. LRG1 mRNA levels were similarly reduced by 5 weeks of carbamazepine, but not valproate administration. Sequence analysis and search of the GenBank database revealed that LRG1 is analogous to the sequence of the gene for rat aldolase A. Conclusions: These results demonstrate that chronic administration of lithium, but not short-term administration, downregulates the levels of aldolase A mRNA, suggesting this effect may play a role in mediating the therapeutic action of this agent.


http://www.sciencedirect.com/science/article/B6T4S-3WJ6WN8-C/2/94c6ad7dbd48920fd3a6a6daa57989a6

Background: Obsessive-compulsive disorder (OCD) is a common and severe psychiatric illness that affects 1-3% of the population and presents a well-established co-morbidity with major depressive disorder (MDD). Twin and family studies have suggested a genetic component in the etiology of OCD, although the mode of inheritance is unknown. Pharmacotherapy of the disease implicates both serotonergic and dopaminergic pathways. Previously, guided by the 22q11 microdeletion-related psychiatric phenotype, we provided evidence for a sexually dimorphic association between OCD and the gene for catechol-O-methyltransferase (COMT). In this report, we use 110 nuclear OCD families to analyze the inheritance of variants of COMT and monoamine oxidase-A (MAOA), another gene modulating monoamine metabolism. Methods: A sample of 110
nuclear OCD families was collected, and lifetime diagnoses were ascertained using the Diagnostic Interview for Genetic Studies (DIGS). DNA was genotyped for functional variants of the COMT and MAO genes, and allele inheritance was examined using the Transmission Disequilibrium Test (TDT) and Haplotype-based Haplotype Relative Risk (HHRR) test. Results: We provide evidence supporting the previously reported sexually dimorphic association between low COMT enzymatic activity and OCD. We also provide evidence for a similar sexually dimorphic association between OCD and an allele of the MAOA gene, previously linked to high MAO-A enzymatic activity. In agreement with the well-established action of MAO-A inhibitors as antidepressants, this association is particularly marked among male OCD probands with co-morbid MDD, who represent more than 50% of our male OCD sample. Conclusions: Our analysis indicates that variants of two genes modulating monoamine metabolism contribute significantly to OCD susceptibility. Most importantly, an unexpected sexually dimorphic pattern of genetic susceptibility to OCD is revealed and suggests the possibility that profound gender differences in genetic predisposition may exist not only for other OCD susceptibility genes, but for an array of other psychiatric disorders as well.


http://www.sciencedirect.com/science/article/B6T4S-47DPS5T-4/2/a31b4e6139c3befaea5beba2f67e4e8f4

BackgroundBased on the dopamine hypothesis, the dopamine D1 receptor gene (DRD1) is considered to be a good candidate gene for bipolar disorder (BP). Methods In our study, three polymorphisms of the DRD1 gene, -800T/C, -48A/G, and 1403T/C, were analyzed in 286 BP trios. Both the transmission disequilibrium test (TDT) and haplotype TDT were performed on the genotype data to test for the presence of linkage disequilibrium between DRD1 and bipolar disorder. With the extended transmission disequilibrium test (ETDT), we also calculated the maternal transmission and paternal transmission for each allele. ResultsAlthough no association was found for each individual polymorphism, there is a significant association between DRD1 and BP for haplotype TDT analysis ($\chi^2 = 16.068, df = 3, p =.0011$). Conclusions These results indicate that DRD1 may play a role in the etiology of bipolar disorder.


http://www.sciencedirect.com/science/article/B6T4S-49MX2KJ-D/2/d70a2c88958f79dab6d2dca9cdffabf

BackgroundBehavioral inhibition to the unfamiliar (BI), a heritable temperamental profile involving an avoidant response to novel situations, may be an intermediate phenotype in the development of anxiety disorders. Corticotropin-releasing hormone (CRH) is a key mediator of the stress response through its effects on the hypothalamic-pituitary-adrenal axis and limbic brain systems. Transgenic mice overexpressing CRH exhibit BI-like behaviors, implicating this gene in the development of the phenotype. Methods We genotyped a marker tightly linked to the CRH locus in 85 families of children who underwent laboratory-based behavioral assessments of BI and performed family-based association analyses. Results We observed an association between an allele of the CRH-linked locus and BI ($p =.015$). Among offspring of parents with panic disorder, this association was particularly marked ($p =.0009$). We further demonstrate linkage disequilibrium between this marker and single nucleotide polymorphisms encompassing the CRH gene. Conclusions These results are consistent with the possibility that variants in the CRH gene are associated with anxiety proneness.

http://www.sciencedirect.com/science/article/B6T4S-3WY9RW4-3/2/30326eaac93428eb85b32e02c206373c

Background: The goal of the current study was to explore the clinical, neuropathological, and neurochemical correlates of the DXS1047 202bp allele in a group of 50 autopsy-confirmed cases of Alzheimer's disease (AD) who lacked other concomitant brain diseases. We previously published the results of a genome survey for novel risk loci for typical-onset (>= 60 years) AD conducted at 10cM resolution (Zubenko et al. 1998a, b). This survey detected associations of alleles at six microsatellite loci with AD, including the 202bp allele of the DXS1047 locus that resides within Xq25 on the human cytogenetic map. Methods: Clinical assessments were performed as part of a longitudinal study of AD and related disorders. Autopsies were performed using standardized methods and the resulting diagnoses were made according to established criteria. Genotyping, morphometry, and neurochemical analyses were performed using postmortem brain tissue. Results: Patients with AD who carried the DXS1047 202bp allele manifested cortical norepinephrine levels that ranged from 2.1 to 3.6 times the corresponding values for noncarriers (p = .002), controlling for the potential effects of gender, age at symptomatic onset or death, and postmortem interval. In contrast, carriers tended to have lower cortical levels of dopamine (p = .10). Conclusions: These findings support the results of our previous genome survey and suggest that the DXS1047 locus, or a locus in close proximity, modulates biological variables relevant to the pathophysiology of AD. In addition to providing insights into the clinical biology of AD, the characterization of biologically meaningful subtypes, including genotypic subtypes associated with particular neurobiological derangements, may be important to the advancement of experimental therapeutics in AD.


http://www.sciencedirect.com/science/article/B6T4S-3XF07F8-3/2/a8dcc3b7ea136357220d0614167c1d43

Background: In a previous genome survey, we detected associations of alleles at six microsatellite loci with typical-onset AD, including the 234bp allele of the D10S1423 locus. The goal of the current study was to explore the clinical, neuropathological, and neurochemical correlates of the D10S1423 234bp allele in a group of 50 autopsy-confirmed cases of Alzheimer's disease (AD) who lacked other brain diseases. Methods: Clinical assessments were performed as part of a longitudinal study of AD and related disorders. Autopsies were performed using standardized methods and diagnoses were made according to established criteria. Genotyping, morphometry, and neurochemical analyses were performed using postmortem brain tissue. Results: Patients with AD who carried the D10S1423 234bp allele manifested substantial reductions in dopamine levels in all six cortical regions examined. In contrast, carriers tended to have higher concentrations of cortical norepinephrine and revealed a dosage effect of the D10S1423 234bp allele. Conclusions: These findings support the results of our genome survey and suggest that a novel susceptibility gene for AD resides near the D10S1423 locus. The characterization of biologically meaningful subtypes, including genotypic subtypes with particular neurobiological derangements, may be important for the advancement of experimental therapeutics in AD.

http://www.sciencedirect.com/science/article/B6WBS-481MHPP-8/2/bdbd1aa723661f3eb77ea4b2809f86bc

In the 1950s the use of primary rhesus macaque kidney cultures to propagate poliovirus for vaccine production led to the contamination of vaccines with simian virus 40 (SV40). African green monkey kidney (AGMK) cultures free of SV40 were used as an alternative cell substrate for vaccine manufacture. In this study we evaluate oral poliovirus seeds, vaccine bulks and vaccines themselves for the presence of a common contaminant of AGMK cultures, simian cytomegalovirus (SCMV). Using sensitive polymerase chain reaction (PCR) techniques, nearly half of the samples analysed were found to be contaminated with SCMV sequences. However, vaccine bulks, positive by PCR for SCMV failed to show any evidence of infectious virus in these studies. One poliovirus vaccine and one seed, propagated on rhesus macaque kidney cultures were found to be positive for the rhesus monkey CMV by PCR.


http://www.sciencedirect.com/science/article/B758K-4DCM2GK-C/2/5f55d0f02201fea68c88e59deb2ff8d85

Imatinib induces a high complete cytogenetic response (CCR) rate in relapsed chronic myelogenous leukemia. By analyzing minimal residual disease (MRD) under the levels of CCR, we tried to assess the molecular response after imatinib therapy. By using real-time quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR), MRD was evaluated in 23 patients (3 in cytogenetic relapse, 6 in chronic phase, 9 in accelerated phase, and 5 in blast crisis) who were treated with standard-dose imatinib for relapsed chronic myelogenous leukemia after allogeneic stem cell transplantation. With a median therapy time of 399 days (range, 35-817 days), 19 (83%) patients achieved a CCR. Meanwhile, 11 (58%) of them achieved a molecular remission (MR), which was associated with improved survival. The Q-RT-PCR data were compared according to the best response (MR, n = 11; CCR, n = 8) in the patients achieving a CCR. The BCR-ABL/ABL ratios were similar in 2 groups at 3 months but were significantly different at 6 months (median, 0.0000012 for MR and 0.00022 for CCR; P =.003). The probability of a subsequent MR was significantly higher in patients with a lower BCR-ABL/ABL ratio at 6 months (100% for P =.006) or a greater reduction in the level between 3 and 6 months (log-reduction [greater-than-or-equal]1.0, 100%; P =.003). Q-RT-PCR is a reliable method for monitoring MRD: the early trends in the BCR-ABL/ABL ratio may be clinically useful in
discriminating patients who will achieve an MR from those who will remain in CCR.


http://www.sciencedirect.com/science/article/B758K-4BV4J4S-7/2/9b6ccf39af1ca796e430d526f6db3001

Donor lymphocyte infusion (DLI) results in complete cytogenetic remission (CCR) of relapsed chronic-phase chronic myeloid leukemia (CML-CP) after allogeneic stem cell transplantation (SCT) in up to 80% of patients. The main complication of DLI is graft-versus-host disease (GVHD). Decreasing the dose of DLI is associated with less GVHD but also with a longer interval between treatment and CCR. We postulated that combining [alpha]-interferon ([alpha]-IFN) with DLI would enable us to decrease the dose of DLI, thereby limiting GVHD, and at the same time to decrease the interval between DLI and CCR for patients with either a hematologic or cytogenetic relapse. For molecular relapses, we hypothesized that because of a lower tumor load, very low doses of DLI without [alpha]-IFN could be an effective treatment. Two groups of CML-CP patients treated with DLI at a very low dose of 0.5 to 1.0 x 10^7 mononuclear cells per kilogram, containing 2 to 6 x 10^6 CD3+ T cells per kilogram, were analyzed: 13 patients with a cytogenetic or a hematologic relapse after allogeneic SCT (group A) were treated with additional [alpha]-IFN therapy at a dose of 3 x 10^6 U 5 d/wk, and 8 patients with a molecular relapse were treated without [alpha]-IFN (group B). Twelve patients from group A reached a CCR. The median interval between DLI and CCR was 7 weeks (range, 5-18 weeks) for group A. All patients with a CCR reached complete donor chimerism at a median of 10 weeks after DLI (range, 6-121 weeks). Eleven patients reached molecular remission at a median of 15 weeks after DLI (range, 8-34 weeks). In group B, all patients reached a molecular remission at a median of 14 weeks (range, 12-29 weeks). Five patients from group A developed acute GVHD grade II to IV and extensive chronic GVHD. In group B, 1 patient developed acute GVHD grade II to IV and subsequently developed extensive chronic GVHD. In group B, 1 patient developed acute GVHD grade II to IV and subsequently developed extensive chronic GVHD. With a median follow-up of 62 months, 10 patients in group A are alive and in continuous CCR. One patient had a molecular relapse, for which she successfully received additional DLI; another patient reached molecular remission only after 5 doses of DLI. Two patients from group A died of a gram-negative sepsis, and 1 died of an acute myocardial infection. In group B, all patients are alive and in molecular remission with a median follow-up of 20 months. One patient's disease progressed but was successfully treated with DLI plus [alpha]-IFN. In conclusion, very-low-dose DLI in combination with [alpha]-IFN as treatment for cytogenetic or hematologic relapses of CML-CP after allogeneic SCT reduced the interval to obtain a CCR with acceptable GVHD when compared with the literature. Patients with a CCR also reached complete donor chimerism and complete molecular remissions. For patients with a molecular relapse, very-low-dose DLI alone is sufficient to induce molecular remissions in most patients and is associated with limited GVHD.

Biology of the Cell (1)

We previously reported that when deprived of fibroblast growth factor, human umbilical vein endothelium-derived cells (HUVE-DCs) are capable of differentiating into smooth muscle-like cells through activin A-induced, Smad-dependent signaling, and that maintenance of the endothelial-cell phenotype and differentiation into smooth muscle-like cells are reciprocally controlled by fibroblast growth factor-1 and activin A (Ishisaki et al., 2003). Here, we examined how protein kinase C (PKC), which plays pivotal roles in the regulation of cellular proliferation and differentiation in numerous cell types, might affect the above differentiation. We found that phorbol-12-myristate-13-acetate-induced down-regulations of some PKCs accompany suppressions of the expressions of smooth muscle cell markers in HUVE-DCs deprived of fibroblast growth factor. Moreover, the PKC-inhibitors Go6850 and Go6983 suppressed the differentiation of HUVE-DCs into smooth muscle-like cells. These results strongly suggest that activation of PKC is involved in the above differentiation.


Transgenic poplar lines were developed to investigate the role of a proteinase inhibitor in pest resistance of woody plants. Using an Agrobacterium binary vector system, the clone 'Hansen' (Populus alba L. x P. grandidentata Michx.) was transformed with chimeric genes containing the coding region of potato proteinase inhibitor II (PIN2) linked to either a bacterial nopaline synthase (nos) or a cauliflower mosaic virus (35S) promoter. All transferred DNA also contained a selectable marker in the form of a nos promoter linked to a neomycin phosphotransferase II (NPT II) structural gene. The presence of the transferred PIN2 and NPT II sequences in poplar was confirmed for nine transgenic lines using polymerase chain reaction (PCR). Expression of PIN2 in leaves of transgenic poplar was demonstrated by enzyme-linked immunosorbent assays (ELISAs) and western blots. Two unique polypeptides from transgenic poplar, of ca 8 kDa and ca 12 kDa, indicate that PIN2 was translated appropriately. Resistance to the imported willow leaf beetle was tested in laboratory bioassays. The untransformed clone 'Hansen' and 11 transgenic lines were submitted to freshly hatched larvae to determine effects on pupal weight, larval development time and leaf area consumed. A significant difference from the untransformed clone in leaf area consumed was detected in one transgenic line, Tr665. Trends were indicated for several other transgenic lines for the other parameters.
Chondrocytes undergo a process of dedifferentiation in monolayer culture that is characterized by a transition to a fibroblast-like phenotype. This behavioral change poses a challenge for tissue-engineered cartilage constructs, as approaches using autologous cells require expansion in vitro. Because chondrocytes express a variety of integrin receptors specific to different adhesive proteins, we hypothesized that chondrocytes expanded on various underlying protein monolayers would have different phenotypic responses. Bovine articular chondrocytes were cultured for up to 2 weeks on tissue culture plastic, fibronectin, collagen type I or collagen type II substrate in the presence or absence of ascorbate. Contrary to our hypothesis, the extracellular matrix protein substrates used in this study did not significantly alter the changes in chondrocyte morphology, gene expression, matrix formation, or cytoskeletal organization. Cells on all substrates assembled equivalent matrices, which may have subsequently regulated cell behavior. In cultures with ascorbate, populations of round and spread cells emerged after 1 week, with round cells expressing collagen type II and the differentiated phenotype and spread cells dedifferentiating. In cultures without ascorbate, chondrocytes rapidly adhered and spread onto organized fibronectin matrices via the [alpha]5[beta]1 integrin, which has been associated with survival and proliferation of chondrocytes in vitro. These findings indicate that expanding chondrocytes on protein monolayers may not be an effective solution to preventing dedifferentiation and improving autologous chondrocyte transplantation.

The re-differentiation capacities of human articular and chick embryo sternal chondrocytes were evaluated by culture on HYAFF-11 and its sulphate derivative, HYAFF-11-S, polymers derived from the benzyl esterification of hyaluronate. Initial results showed that the HYAFF-11-S material promoted the highest rate of chondrocyte proliferation. RNA isolated from human and chick embryo chondrocytes cultured in Petri dishes, HYAFF-11 or HYAFF-11-S were subjected to semi-quantitative RT-PCR analyses. Human collagen types I, II, X, human Sox9 and aggrecan, chick collagen types I, II, IX and X were analysed. Results showed that human collagen type II mRNA expression was upregulated on HYAFF-11 biomaterials. In particular, a high level of collagen type IIB expression was associated with three-dimensional culture conditions, and the HYAFF-11 material was the most supportive for human collagen type X mRNA expression. Human Sox9 mRNA levels were constantly maintained in monolayer cell culture conditions over a period of 21 days, while these were upregulated when chondrocytes were cultured on HYAFF-11 and HYAFF-11S. Furthermore, chick collagen type IIA and IIB mRNA expression was detected after only 7 days of HYAFF-11 culture. Chick collagen type IX mRNA expression decreased in scaffold cultures over time. Histochemical staining performed in engineered cartilage revealed the presence of a de novo synthesized glycosaminoglycan-rich extracellular matrix; immunohistochemistry confirmed the deposition of collagen type II. This study showed that the three-dimensional HYAFF-11 culture system is both an effective chondrocyte delivery system for the treatment of articular cartilage defects, and an excellent in vitro model for studying cartilage differentiation.

http://www.sciencedirect.com/science/article/B6TWB-4FF8WM6-1/2/940ecce02898e3754459b641d024265e

Functional engineering of musculoskeletal tissues generally involves the use of differentiated or progenitor cells seeded with specific growth factors in biomaterial scaffolds. Ideally, the scaffold should be a functional and structural biomimetic of the native extracellular matrix and support multiple tissue morphogenesis. We have previously shown that electrospun, three-dimensional nanofibrous scaffolds that morphologically resemble collagen fibrils are capable of promoting favorable biological responses from seeded cells, indicative of their potential application for tissue engineering. In this study, we tested a three-dimensional nanofibrous scaffold fabricated from poly(ε-caprolactone) (PCL) for its ability to support and maintain multilineage differentiation of bone marrow-derived human mesenchymal stem cells (hMSCs) in vitro. hMSCs were seeded onto pre-fabricated nanofibrous scaffolds, and were induced to differentiate along adipogenic, chondrogenic, or osteogenic lineages by culturing in specific differentiation media. Histological and scanning electron microscopy observations, gene expression analysis, and immunohistochemical detection of lineage-specific marker molecules confirmed the formation of three-dimensional constructs containing cells differentiated into the specified cell types. These results suggest that the PCL-based nanofibrous scaffold is a promising candidate scaffold for cell-based, multiphasic tissue engineering.


http://www.sciencedirect.com/science/article/B6TWB-4DDXRBT-4/2/8b7451687d7c8f614d2379c296dfdd522

Mineralized and partially or fully demineralized biomaterials derived from bovine bone matrix were evaluated for their ability to support human bone marrow stromal cell (BMSC) osteogenic differentiation in vitro and bone-forming capacity in vivo in order to assess their potential use in clinical tissue-engineering strategies. BMSCs were either seeded on bone-derived scaffolds and cocultured in direct cell-to-scaffold contact, allowing for the exposure of soluble and insoluble matrix-incorporated factors, or cocultured with the scaffold preparations in a transwell system, exposing them to soluble matrix-incorporated factors alone. Osteoblast-related markers, alkaline phosphatase (ALP) activity and bone sialoprotein (BSP) and osteopontin (OP) mRNA expression were evaluated in BMSCs following 14 days of cocultivation in both systems. The data demonstrate that BMSCs from some donors express significantly higher levels of all osteoblast-related markers following cocultivation in direct cell-to-scaffold contact with mineralized scaffolds in comparison to fully demineralized preparations, while BMSCs from other donors display no significant differences in response to various scaffold preparations. In contrast, BMSCs cocultured independently with soluble matrix-incorporated factors derived from each scaffold preparation displayed significantly lower levels of ALP activity and BSP mRNA expression in comparison to untreated controls, while no significant differences were observed in marker levels between cells cocultured similarly with different biomaterial preparations. In addition, BMSCs were seeded directly on mineralized and partially or fully demineralized biomaterials and implanted in subcutaneous sites of athymic mice for 8 weeks to evaluate their in vivo bone-forming capacity. The ex vivo incorporation of BMSCs into all bone-derived scaffold preparations substantially increased the mean extent and frequency of samples containing de novo bone formation over similar nonseeded controls, as determined by histological and
histomorphometrical analysis. No statistically significant differences were observed in the extent or frequency of bone formation between various scaffold preparations seeded with BMSCs from different donors. These results demonstrate that the in vivo osteoinductivity of bone-derived scaffolds can be modulated by ex vivo incorporated BMSCs and the extent of scaffold demineralization plays a significant role in influencing in vitro osteogenic differentiation of BMSCs depending on the coculture system and BMSC donor.


http://www.sciencedirect.com/science/article/B6TWB-4B9D7R3-1/2/d3aa4dd7c98696d154b54b4adfd3cc

During prolonged cultivation ex vivo, adult bone marrow stromal stem cells (BMSCs) undergo two probably interdependent processes, replicative aging and a decline in differentiation potential. Recently, our results with primary human fibroblasts indicated that growth on denatured collagen (DC) matrix results in the reduction of the rate of cellular aging. The present study has been undertaken to test whether the growth of human BMSCs under the same conditions would translate into preservation of cellular aging-attenuated functions, such as the ability to express HSP70 in response to stress as well as of osteogenic differentiation potential. We report here that growth of BMSCs on a DC matrix versus tissue culture polystyrene significantly reduced one of the main manifestations of cellular aging, the attenuation of the ability to express a major protective stress response component, HSP70, increased the proliferation capacity of ex vivo expanded BMSCs, reduced the rate of morphological changes, and resulted in a dramatic increase in the retention of the potential to express osteogenic-specific functions and markers upon treatment with osteogenic stimulants. BMSCs are a promising and increasingly important cell source for tissue engineering as well as cell and gene therapeutic strategies. For use of BMSCs in these applications, ex vivo expansion is necessary to obtain a sufficient, therapeutically useful, number of cells; however, this results in the loss of differentiation potential. This problem is especially acute in older patients where more extensive in vitro expansion of smaller number of stem/progenitor cells is needed. The finding that growth on certain biomaterials preserves aging-attenuated functions, enhances proliferation capacity, and maintains differentiation potential of BMSCs indicates a promising approach to address this problem.


http://www.sciencedirect.com/science/article/B6TWB-4D2FKCN-4/2/45910fa9c40074db9e4371be7cbb8d41

We investigated whether the post-expansion redifferentiation and cartilage tissue formation capacity of adult human nasal chondrocytes can be regulated by controlled modifications of scaffold composition and architecture. As a model system, we used poly(ethylene glycol)-terephthalate-poly(butylene)-terephthalate block copolymer scaffolds from two compositions (low or high PEG content, resulting in different wettability) and two architectures (generated by compression molding or three-dimensional (3D) fiber deposition) with similar porosity and mechanical properties, but different interconnecting pore architectures. Scaffolds were seeded with expanded human chondrocytes and the resulting constructs assessed immunohistochemically, biochemically and at the mRNA expression level following up to 4 weeks of static culture. For a given 3D architecture, the more hydrophilic scaffold enhanced cell redifferentiation and cartilaginous tissue formation after 4 weeks culture, as assessed by higher
mRNA expression of collagen type II, increased deposition of glycosaminoglycan (GAG) and predominance of type II over type I collagen immunostain. The fiber-deposited scaffolds, with a more accessible pore volume and larger interconnecting pores, supported increased GAG deposition, but only if a more hydrophilic composition was used. By applying controlled and selective modifications of chemico-physical scaffold parameters, we demonstrate that both scaffold composition and architecture are instructive for expanded human chondrocytes in the generation of 3D cartilaginous tissues. The observed effects of composition and architecture were likely to have been mediated, respectively, by differential serum protein adsorption and efficiency of nutrient/waste exchange.


http://www.sciencedirect.com/science/article/B6TWB-47DH8T7-J/2/b03e0c58ef6fedf36d241cfc22e36fde

This report completes a previous study on the growth and metabolism of fetal bovine epiphyseal chondrocytes cultured, within native or cross-linked collagen sponges carried out without the addition of fresh ascorbate. At low initial cell density (2.3 x 10^6 cells/cm^3) cell proliferation and a low matrix deposition were observed, whereas at high initial cell density (2.3 x 10^7 cells/cm^3) there was an absence of cell proliferation, but the deposition of a cartilage-like matrix was measured. In both cases, only traces of type I collagen (marker of chondrocyte dedifferentiation) were detected. In this report, we observed, after 1 month in culture with ascorbate, in both type of scaffolds and initial cell densities, an increase in cell proliferation (2-fold) and in expression of genes encoding for collagen types I, II, X and MMP-2 and -13, but no change in the level of matrix deposition (collagen and GAG). With regard to the proteins present, the main differences with or without ascorbate concerned the increase of neosynthesised type I collagen (up to 35% of the total collagen deposited in the sponge) and of the MMP-2 active form. In conclusion, these results show that ascorbate is an important factor to consider when preparing cartilage constructs for its action on chondrocyte phenotype modulation and proliferation.


http://www.sciencedirect.com/science/article/B6TWB-418PMNJ-C/2/96f7d5405188f0832690164b62d083f

It has been demonstrated that using cultured chondrocytes that have been seeded onto various biomatrices can enhance the quality of the articular cartilage repair tissue. As tissue-engineering becomes increasingly more complex there is a need to understand how a specific biomaterial may influence gene expression. In this study several commonly used scaffold materials for cartilage tissue engineering were evaluated with respect to their influence on matrix gene expression. Primary cultures of bovine chondrocytes were established in monolayer then seeded onto polylactic acid (PLLA), polyglycolic acid (PGA), collagen matrices. The induction of collagen type I, collagen type II, and aggrecan was observed at various time points on these biomaterials using RT-PCR. The collagen type I gene was upregulated on collagen scaffolds throughout the culture period. PLLA and PGA showed initial induction followed by downregulation. Monolayer culture did not induce collagen I message. Collagen II genes were selectively upregulated after 72 and 96 h post seeding depending the scaffold material. Monolayer culture had strong induction of collagen II. The aggrecan protein was consistently expressed in all scaffold materials cultures and monolayer.

http://www.sciencedirect.com/science/article/B6TWB-44FCYG3-T/2/eb79b04e766c8278ac8f7abad573c2d

An in vivo model of the inflammatory response to orthopaedic biomaterials was used to examine cellular and cytokine responses to polymer particles of ultra high molecular weight polyethylene (UHMWPE) and polymethylmethacrylate (PMMA), and metal particles of cobalt-chrome (Co-Cr) and titanium alloy (Ti-6Al-4V). Responses were determined separately and in combinations, to examine interactions between different forms of biomaterials. Murine air pouches were injected with particle suspensions, and reactions evaluated using histological, immunological, and molecular techniques. All particulate biomaterials caused significant increases in membrane thickness compared with control (saline) air pouches, with the highest reaction seen in response to Ti-6Al-4V particles. A synergistic increase in membrane thickness was observed when PMMA was combined with UHMWPE, suggesting that multiple biomaterial stimuli markedly increase the inflammatory reaction. Cellular analysis indicated that all particles increased the absolute number and the percentage of macrophages in the membrane over the control level, with the most pronounced increase due to individual biomaterial occurring with UHMWPE particles. Cytokine analysis revealed that biomaterials provoked a strong IL-1 response. Ti-6Al-4V stimulated the highest IL-6 gene transcription and the lowest IL-1 gene transcription. The data suggest that synergism in the inflammatory response to biomaterials may be important in adverse responses to orthopaedic wear debris.


http://www.sciencedirect.com/science/article/B6TWB-45CW107-2/2/3afe396c3697f793d283bef2b3ebb82a

The wear of orthopaedic prostheses results in the release of a markedly heterogeneous assortment of particulate debris, with respect to both size and shape. Although particle size has been extensively examined, the role of particle shape in adverse inflammatory reactions to debris remains unclear. Using an in vivo murine model of inflammation, we assessed tissue responses to globular and to elongated ultra-high molecular weight polyethylene (UHMWPE) particles with a similar surface area, and investigated whether inflammation and cellular apoptosis varied with particle shape in the debris-tissue interaction. Histological changes of UHMWPE-stimulated pouch membrane were assessed using a computerized image analysis system. Quantitative real time PCR and ELISA were performed to assess mRNA expression and protein level of the cytokines, and TUNEL assays were conducted to quantify apoptotic cells. The data revealed that elongated particles generated more active inflammatory air pouches, stimulated more severe membrane proliferation and the inflammatory cellular infiltration compared to globular particles. Increased levels of IL-1[beta] and TNF[alpha] were detected in the lavage and homogenate of pouches stimulated with elongated particles in comparison to pouches with globular particles, and the apoptotic assay indicated more severe apoptotic changes within the inflammatory membrane provoked with elongated particles. Our results suggest that cellular responses to UHMWPE wear debris are dependent on the shape of the particles.

Improvement of the biochemical characteristics of enzymes has been aided by misincorporation mutagenesis and DNA shuffling. Many gene shuffling techniques result predominantly in the regeneration of unshuffled (parental) molecules. We describe a procedure for gene shuffling using degenerate primers that allows control of the relative levels of recombination between the genes that are shuffled, and reduces the regeneration of unshuffled parental genes. This shuffling procedure avoids the use of endonucleases for gene fragmentation prior to shuffling and allows the inclusion of random mutagenesis of selected portions of the chimeric genes as part of the procedure. We illustrate the use of the shuffling technique with a family of [beta]-xylanase genes that possess widely different G + C contents. In addition, we introduce a new method (RNDM) for rapid screening of mutants from libraries where no adaptive selection has been imposed on the cells. They are identified only by their retention of enzymatic activity. The combination of RNDM followed by DOGS allows a comprehensive exploration of a protein's functional sequence space.


Efficient and sustained transgene expression are desirable features for many envisioned gene therapy applications, yet synthetic vectors tested to date are rarely successful in achieving these properties. Substantial research efforts have focused on protection of plasmid DNA from nuclease attack as well as increasing nuclear transport of plasmids, resulting in significant but still limited gains. We show here that a further barrier to efficient and sustained expression exists for synthetic vectors: plasmid DNA methylation. We have investigated this barrier for transient expression of a green fluorescent protein (GFP) transgene delivered via Lipofectamine, by testing the effects of culturing C3A human hepatoblastoma cells with 5-Azacytidine (AzaC), an irreversible inhibitor of DNA methyltransferase. To control for loss of plasmids by dilution during mitosis, transfected cells were growth-arrested for 1 week and their subsequent GFP expression quantified by FACS. In the presence of AzaC, a significantly greater fraction of transfected cells remained GFP-positive and possessed higher levels of GFP production relative to AzaC-untreated cells. Additionally, we have applied a Methyl-Assisted PCR (MAP) assay to quantify a subset of methylated CpG sites in the GFP gene. When MAP was performed on plasmids isolated from transfected cells, the extent of methylation was found to be inversely related to the level of GFP expression.

The utility of using genomic DNA directly in agarose, i.e. cloneless libraries, in place of large clone libraries, radiation hybrid panels, or chromosome dissection was demonstrated. The advantage of the cloneless library approach is that, in principle, a targeted genomic resource can be developed rapidly for any genomic region using any genomic DNA sample. Here, a human chromosome 20 Not I fragment library was generated by slicing a pulsed field gel lane containing fractionating Not I cleaved DNA from a monosomic hybrid cell line into 2 mm pieces. A reliable PCR method using agarose embedded DNA was developed. InterAlu PCR generated unique patterns of products from adjacent slices (e.g. fractions). Further, the specificity of the interAlu products was demonstrated by FISH analysis and in other hybridization experiments to arrayed interAlu products. STS content mapping was used to order the fractions and also demonstrate the unique content of the library fractions.


http://www.sciencedirect.com/science/article/B6VRM-41FKWH0-1/2/22d66c90aacf73276110ea6ff08e381f

cDNA representational difference analysis (cDNA RDA) is a PCR-based subtractive enrichment procedure for the cloning of differentially expressed genes. In this study, we have further developed the procedure to take advantage of solid-phase technology, and to facilitate the use of RDA when starting material is limited. Several parameters of the PCR-based generation of cDNA representations were investigated, and a solid-phase based purification step was introduced to simplify removal of digested adapter-ends and uncleaved fragments. The use of magnetic particles increased the speed of the method, and also eliminated the risk of carry-over contamination between iterative steps of subtraction and PCR amplification. The modified protocol was evaluated in monitoring differences in gene expression in (i) a rat system consisting of livers with and without growth hormone treatment, and in (ii) a human system consisting of normal colon and colon cancer.

Bioorganic & Medicinal Chemistry (2)


http://www.sciencedirect.com/science/article/B6TF8-49FGJTF-B/2/315f6ba7ac71d8539962c90aabd121cd

Hairpin polyamides selectively recognize predetermined DNA sequences with affinities comparable to naturally occurring proteins. Internal side-by-side pairs of unsymmetrical aromatic rings within the minor groove of DNA distinguish each of the four Watson-Crick base pairs. In contrast, N-terminal ring pairs exhibit less specificity, with the exception of Im/Py targeting G.C base pairs. In an effort to explore the sequence specificity of new ring pairs, a series of hairpin polyamides containing 3-substituted-thiophene-2-carboxamide residues at the N-terminus was synthesized. An N-terminal 3-methoxy (or 3-chloro) thiophene residue paired opposite Py displayed 6- (and 3-) fold selectivity for T.A relative to A.T base pair, while disfavoring G,C base
pairs by >200-fold. Our data suggests shape selective recognition with projection of the 3-thiophene substituent (methoxy or chloro) to the floor of the minor groove.


http://www.sciencedirect.com/science/article/B6TF8-48W2M9W-4/2/7086939f869a52fbd9c8a1804d2db3a6

Cloning of polyether polyketide synthase (PKS) genes for salinomycin biosynthesis was attempted from Streptomyces albus. Seven [beta]-ketoacyl synthase (KS) core regions were obtained by PCR amplification using primers designed based on the conserved KS domains of type I PKSs. Using the KS fragment as a probe, screening of an S. albus genomic DNA library was carried out by colony hybridization. From the positive cosmide clone isolated, a 4.5-kb BamHI fragment was subcloned and sequenced. It showed high homology with bacterial type I PKSs and was deduced to code for KS, malonyl transferase, and ketoreductase motifs. By gene disruption with this 4.5-kb BamHI fragment, the cloned gene was shown to be a part of the salinomycin biosynthetic gene cluster of S. albus.

Bioorganic & Medicinal Chemistry Letters (2)


http://www.sciencedirect.com/science/article/B6TF9-3YRV9TB-5/2/37f13684dc0916d1eb3b88f0b87dba3e9

We have succeeded in the acquisition of DNA aptamers that recognize chitin using in vitro selection. The obtained DNA aptamers have the stem-loop or bulge loop structures with guanine rich loop clusters and the clockwise B-form stems.


http://www.sciencedirect.com/science/article/B6TF9-3SFNS9Y-M/2/da7ebd62f77308773d5a5daddeec5b5f

The acyclic nucleoside triphosphates 1 and 2 were prepared and tested as substrates for several DNA replicating enzymes; AmpliTaq(R) FS and Taquenase(R) accepted these compounds as substrates leading to chain termination.

http://www.sciencedirect.com/science/article/B6V24-4BH67T9-3/2/0fb79c6b00649cb5604600f783c96c574

A laboratory-scale continuously stirred anaerobic thermophilic batch digester was inoculated with cattle manure. Bacterial and archaeal communities, as well as digester performances, were analysed during reactor start-up for about 20 days. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) was used for overall detection and for study of the dynamics of microbial populations. Dominant bacteria and archaea 16S rDNAs were sequenced from the sample on day 12. Ten bacteria and 3 archaea OTUs (operational taxonomic units) were identified from the 52 clones sequenced. Sequences corresponding to the dominant bacterial SSCP peak were phylogenetically close to the 16S rDNA sequence of Bacillus thermoterrestris, whereas sequences corresponding to the two dominant archaeal SSCP peaks were phylogenetically close to the 16S rDNA sequence of Methanoculleus thermophilicus and Methanosarcina thermophila.


To clarify the characteristics of thermophilic bacteria in cattle manure compost, enzymatic activity and species diversity of cultivated bacteria were investigated at 54, 60, 63, 66 and 70 [deg]C, which were dependent on composting temperature. The highest level of thermophilic bacterial activity was observed at 54 [deg]C. Following an increase in temperature to 63 [deg]C, a reduction in bacterial diversity was observed. At 66 [deg]C, bacterial diversity increased again, and diverse bacteria including Thermus spp. and thermophilic Bacillus spp. appeared to adapt to the higher temperature. At 70 [deg]C, bacterial activity measured as superoxide dismutase and catalase activity was significantly higher than at 66 [deg]C. However, the decomposition rate of protein in the compost was lower than the rate at 66 [deg]C due to the higher compost temperature.


http://www.sciencedirect.com/science/article/B6TFC-447MWX3-N/2/afa3166e38d38198a7a8c1090dd50ddf
Rapid, sensitive assays for nucleic acid amplification products have utility for the identification of bacterial or viral infections. We have developed a nucleic acid hybridization assay utilizing thin film technology that permits visual detection of hybrids. The silicon-based biosensor detects the presence of target sequences by enzymatically transducing the formation of nucleic acid hybrids into molecular thin films. These films alter the interference pattern of light on the biosensor surface, producing a perceived color change. We have applied this technology to the development of a chip containing capture probes specific for human respiratory virus sequences including respiratory syncytial virus, influenza virus A and B, parainfluenza virus types 1 and 3, and rhinovirus. In a ten-minute assay, the biosensor permits unambiguous identification of virus-specific RT/PCR products from infected cell lysates.

Kramer, K., M. Fiedler, et al. (2002). "A generic strategy for subcloning antibody variable regions from the scFv phage display vector pCANTAB 5 E into pASK85 permits the economical production of Fab fragments and leads to improved recombinant immunoglobulin stability." Biosensors and Bioelectronics 17(4): 305.

http://www.sciencedirect.com/science/article/B6TFC-453FYR0-8/2/0823f1f42bcf3ae5f7f9fdeae7a47c71

Apart from the decisive sensitivity and specificity of immuno-sensors, the employed antibodies essentially contribute to additional key factors like fabrication costs for sensor chips and sensor stability. A production scheme for recombinant antibody fragments has been optimised with respect to these particular issues of biosensor development. The phagemid vector pCANTAB 5 E is widely used for the selection of antibody fragments from corresponding libraries. However, large-scale production of the selected single-chain Fv (scFv) fragments is substantially restricted by the high cost for the inducer IPTG and the anti-E-tag antibody. The latter is needed in significant amounts for the purification of the recombinant protein. A generic strategy was established for subcloning scFv variable regions from pCANTAB 5 E into the plasmid pASK85 for the expression of Fab fragments. pASK85 bears coding sequences for murine constant domains including a His6 tag at the carboxyl-terminal end of the constant heavy chain domain. The anti-s-triazine antibody K47H served as a model system in this study. Biosynthesis of the Fab fragment in a high cell density fermenter was induced by addition of anhydrotetracycline. The Fab fragment was subsequently purified from the periplasmic extract in a single step by immobilized metal affinity chromatography (IMAC). A yield of 100 [μg/l x OD550] purified Fab fragment was obtained employing a standard fermentation scheme. The sensitivity and cross-reactivity of the Fab was comparable to the parent scFv when assayed by enzyme immunoassay. However, the Fab fragment exhibited significantly improved long-term stability.


http://www.sciencedirect.com/science/article/B6TFC-4D3B1X0-1/2/65aaf2f42d676822393498898a5c01f

A novel method for DNA quantification and specific sequence detection in a highly integrated silicon microchamber array is described. Polymerase chain reaction (PCR) mixture of only 40 nL volume could be introduced precisely into each chamber of the mineral oil layer coated microarray by using a nanoliter dispensing system. The elimination of carry-over and cross-contamination between microchambers, and multiple DNA amplification and detection by TaqMan chemistry were demonstrated, for the first time, by using our system. Five different gene targets, related to Escherichia coli were amplified and detected simultaneously on the same chip by using
DNA from three different serotypes as the templates. The conventional method of DNA quantification, which depends on the real-time monitoring of variations in fluorescence intensity, was not applied to our system, instead a simple method was established. Counting the number of the microchambers with a high fluorescence signal as a consequence of TaqMan PCR provided the precise quantification of trace amounts of DNA. The initial DNA concentration for Rhesus D (RhD) gene in each microchamber was ranged from 0.4 to 12 copies, and quantification was achieved by observing the changes in the released fluorescence signals of the microchambers on the chip. DNA target could be detected as small as 0.4 copies. The amplified DNA was detected with a CCD camera built-in to a fluorescence microscope, and also evaluated by a DNA microarray scanner with associated software. This simple method of counting the high fluorescence signal released in microchambers as a consequence of TaqMan PCR was further integrated with a portable miniaturized thermal cycler unit. Such a small device is surely a strong candidate for low-cost DNA amplification, and detected as little as 0.4 copies of target DNA.


http://www.sciencedirect.com/science/article/B6TFC-447MWX3-1R/2/994257316bb71f815e7ed10528103e75

Highly integrated hybridization assay and capillary electrophoresis have improved the throughput of DNA analysis. The shift to high throughput analysis requires a high speed DNA amplification system, and several rapid PCR systems have been developed. In these thermal cyclers, the temperature was controlled by effective methodology instead of a large heating/cooling block preventing rapid thermal cycling. In our research, high speed PCR was performed using a silicon-based microchamber array and three heat blocks. The highly integrated microchamber array was fabricated by semiconductor microfabrication techniques. The temperature of the PCR microchamber was controlled by alternating between three heat blocks of different temperature. In general, silicon has excellent thermal conductivity, and the heat capacity is small in the miniaturized sample volume. Hence, the heating/cooling rate was rapid, approximately 16 [deg]C/s. The rapid PCR was therefore completed in 18 min for 40 cycles. The thermal cycle time was reduced to 1/10 of a commercial PCR instrument (Model 9600, PE Applied Biosystems-3 h).


http://www.sciencedirect.com/science/article/B6TFC-4CG2JMP-1/2/9a911d66a8d218b1e09989782c99e9d7

Rapid, accurate, and sensitive detection of biothreat agents requires a broad-spectrum assay capable of discriminating between closely related microbial or viral pathogens. Moreover, in cases where a biological agent release has been identified, forensic analysis demands detailed genetic signature data for accurate strain identification and attribution. To date, nucleic acid sequences have provided the most robust and phylogenetically illuminating signature information. Nucleic acid signature sequences are not often linked to genomic or extrachromosomal determinants of virulence, a link that would further facilitate discrimination between pathogens and closely related species. Inextricably coupling genetic determinants of virulence with highly informative nucleic acid signatures would provide a robust means of identifying human, livestock, and agricultural pathogens. By means of example, we present here an overview of two general applications of microarray-based methods for: (1) the identification of candidate virulence factors; and (2) the analysis of genetic polymorphisms that are coupled to Bacillus anthracis virulence factors using an accurate, low cost solid-phase mini-sequencing assay. We show that microarray-
based analysis of gene expression can identify potential virulence associated genes for use as candidate signature targets, and, further, that microarray-based single nucleotide polymorphism assays provide a robust platform for the detection and identification of signature sequences in a manner independent of the genetic background in which the signature is embedded. We discuss the strategy as a general approach or pipeline for the discovery of virulence-linked nucleic acid signatures for biothreat agents.


http://www.sciencedirect.com/science/article/B6TFC-49P48R1-1/2/607f3a1044059b71ba931fc089341fb8

We designed an automated workstation for magnetic particle-based single nucleotide polymorphism (SNP) discrimination of ALDH genotypes. Bacterial magnetic particles (BMPs) extracted from Magnetospirillum magneticum AMB-1 were used as DNA carriers. The principle for SNP discrimination in this study was based on fluorescence resonance energy transfer (FRET) between FITC (donor) and POPO-3 (acceptor) bound to double-stranded DNA. The workstation is equipped with a 96-way automated pipetter which collects and dispenses fluids as it moves in x- and z-directions. The platform contains a disposable tip rack station, a reagent vessel serving as a stock for POPO-3 and FITC-labeled probes and a reaction station for a 96-well microtiter plate. BMPs were collected by attaching a neodymium iron boron sintered (Nd-Fe-B) magnet on the bottom of the microtiter plate. This system permits the simultaneous heating and magnetic separation of 96 samples per assay. The genotypes ALDH2*1 and ALDH2*2 were discriminated by calculating the relative fluorescence intensities on BMPs.


http://www.sciencedirect.com/science/article/B6TFC-47YPN78-4/2/1792795596f0d8918aaec002252964e2

A single nucleotide polymorphism (SNP) genotyping for aldehyde dehydrogenase 2 gene (ALDH2) has been developed by using a nano-sized magnetic particle, which was synthesized intracellularly by magnetic bacteria. Streptavidin-immobilized on bacterial magnetic particles (BMPs) were prepared using biotin labeled cross-linkers reacting with the amine group on BMPs. ALDH2 fragments from genomic DNA were amplified using a TRITC labeled primer and biotin labeled primer pair, and conjugated onto BMP surface by biotin-streptavidin interaction. PCR product-BMP complex was observed at a single particle level by fluorescence microscopy. These complexes were treated with restriction enzyme, specifically digesting the wild-type sequence of ALDH2 (normal allele of ALDH2). The homozygous (ALDH2*1/*1), heterozygous (ALDH2*1/*2), and mutant (ALDH2*2/*2) genotypes were discriminated by three fluorescence patterns of each particle. SNP genotyping of ALDH2 has been successfully achieved at a single particle level using BMP.

http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-3/2/0e466ea817ae6b33c43ec800ba366d20

Energy dissipation associated with logic operations imposes a fundamental physical limit on computation and is generated by the entropic cost of information erasure, which is a consequence of irreversible logic elements. We show how to encode information in DNA and use DNA amplification to implement a logically reversible gate that comprises a complete set of operators capable of universal computation. We also propose a method using this design to connect, or 'wire', these gates together in a biochemical fashion to create a logic network, allowing complex parallel computations to be executed. The architecture of the system permits highly parallel operations and has properties that resemble well known genetic regulatory systems.


http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-B/2/c664531aadb6b7d80d1c2e861592081f

In our previous paper, we described a method by which a state machine is implemented by a single-stranded DNA molecule whose 3'-end sequence encodes the current state of the machine. Successive state transitions are performed in such a way that the current state is annealed onto an appropriate portion of DNA encoding the transition table of the state machine and the next state is copied to the 3'-end by extension with polymerase. In this paper, we first show that combined with parallel overlap assembly, a single series of successive transitions can solve NP-complete problems. This means that the number of necessary laboratory steps is independent from the problem size. We then report the results of two experiments concerning the implementation of our method. One is on isothermal reactions which greatly increase the efficiency of state transitions compared with reactions controlled by thermal cycles. The other is on the use of unnatural bases for avoiding out-of-frame annealing. The latter result can also be applied to many DNA-based computing paradigms.


http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-R/2/de37dfb0b58f0cd31874b19844cbebf6

DNA computing on surfaces is where complex combinatorial mixtures of DNA molecules are immobilized on a substrate and subsets are tagged and enzymatically modified (DESTROY) in repeated cycles of the DNA computation. A restriction enzyme has been chosen for the surface DESTROY operation. For the READOUT operation, both cycle sequencing and PCR amplification followed by addressed array hybridization were studied to determine the DNA sequences after the computations.
We describe here a quantitative real-time PCR assay for the detection of single-base-pair differences that does not require fluorescently labeled gene-specific probes or complicated primer combinations. Following PCR or RT-PCR of a gene segment that may contain allele-specific differences, 100 pg amplified product are used for a real-time PCR with allele-specific primers and SYBR Green. The use of HEPES buffer at a pH of 6.95 together with AmpliTaq DNA polymerase results in a threshold difference between the correct template and the mismatched template of as many as 20 cycles, depending on the mismatch. Correct matches can be detected in an excess of mismatched template at least at the 0.01 level for the six primer-template matches versus mismatches tested: GC vs. A.C, AT vs. G.T, GC vs. C.C, GC vs. G.G, AT vs. C.T, and GC vs. G.A. Because the initial amplification is separate from real-time detection, conditions can be independently optimized for each step, making the assay particularly suitable for the detection of allele-specific expression in single cells.

BACKGROUND The mechanism(s) of intravenous immunoglobulin (IVIG) towards inhibition of monocyte phagocytic activity involves the function and/or the expression of inhibitory Fc(\gamma)RIIb in a murine model. To confirm these findings in human monocytes, we used a human monocyte phagocytic model in vitro to study the effects of IVIG on the phagocytic activity and the expression of Fc(\gamma)RII genes. METHODS Part A: Monolayer Monocyte Phagocytosis Assay Normal volunteer's peripheral blood mononuclear cells (PBMC) were isolated from heparin anticoagulated blood by Ficoll-Hypaque (Pharmacia Biotech) density separation. The PBMCs were washed and the monocytes were purified using a magnetic bead-positive selection method with anti-CD14 antibody (Miltenyl Biotec). 105 monocytes were incubated in a microtiter plate at 37\degreeC for 1 hour before exposed to IVIG 0.5 g/L. Anti-D (WinRho) sensitized Rh positive (R2R2) red cells were added to the monocytes at 0.5 hour and 18 hour post-IVIG treatment. After 1 hour incubation with sensitized RBC, monocytes phagocytic activity is measured by chemiluminescence detection with a LumiCount (Packard). The readings were normalized with maximal chemiluminescence signal achieved by the monocytes without prior exposure to IVIG (positive control). Part B: RT-PCR of Fc(\gamma)RIIa and Fc(\gamma)RIIb
After 18 hours of exposure to two different concentrations of IVIG (0.5 and 5 gm/L), monocytes were collected and total RNA was isolated with TRizol reagent (Invitrogen). 1 {micro}g of RNA was used to generate first strand cDNA using Superscript II RT kit (Invitrogen). Fc(gamma)RIIa and IIb were amplified with AmpliTaq Gold DNA polymerase system (Applied Biosystems). The PCR products were evaluated by polyacrylamide gel electrophoresis. RESULTS Part A: Dose-response curves were generated by plotting normalized chemiluminescence against the concentration of anti-D used to sensitize the red cells. Anti-D sensitized red cells were phagocytosed by monocytes in a dose-dependent manner. There is a time-dependent inhibition of monocyte phagocytosis when monocytes were incubated with IVIG at 0.5 gm/L. (Fig. 1) Part B: There is no significant difference in the gene expression of FcR(gamma)IIb and Fc(gamma)RIIa in the adherent monocytes after incubating with either low dose (0.5 gm/L) or high dose (5 gm/L) of IVIG for 18 hours. (Fig 2) CONCLUSION Delayed inhibition of phagocytic activity with 18-hour exposure to IVIG is not directly mediated via the modulation of Fc(gamma)RIIb gene expression in human monocytes. Other mechanisms, such as intracellular signalling or receptor coupling, might be involved in the delayed inhibitory effects of IVIG.


http://www.sciencedirect.com/science/article/B6WBV-4DCWF7N-3/2/701e168ff5212444a04306395f955139

HFE-hemochromatosis is the most common form of hereditary hemochromatosis. The disorder is associated with the homozygous C282Y mutation and has variable phenotype, being modulated by environmental and genetic factors. Candidate modifier genes are hemojuvelin and hepcidin, which are responsible for juvenile hemochromatosis. We used DHPLC to scan mutations in these genes in a cohort of unrelated patients with C282Y mutation. They consisted of 136 C282Y homozygous, 43 heterozygous, and 42 C282Y/H63D compound heterozygous, plus 62 controls subjects. Mutations and polymorphisms were found in 16 patients and 4 controls. Abnormally high indices of iron status were found in subjects C282Y/H63D heterozygous for the N196K hemojuvelin mutation and the -72C>T hepcidin substitution. The already described G71D mutation of hepcidin did not induce evident modification of the C282Y/H63D phenotype. The data show that heterozygous mutations of the hemojuvelin gene contribute like those of hepcidin to the phenotypic heterogeneity of hemochromatosis. However, they are rare and explain only a minor portion of the variable penetrance of the disorder.

Iron overload was found to be the major cause of disability in Chinese HbH disease patients although they were not on regular blood transfusion. The transferrin receptor 2 (TFR2) and hereditary hemochromatosis (HFE) genes were examined to see if inheritance of these gene defects may be a possible cause of iron overload in 45 HbH patients. A novel intronic (IVS6 (+6) T->A) mutation of the TFR2 gene was identified in one patient, and six others were found to carry a known missense mutation (exon 5, I238M) that was also present in one normal control subject. One HbH patient and one normal control carried the H63D mutation of the HFE gene. Since only eight out of 45 iron-overloaded HbH patients carry a defect in the TFR2 or HFE gene in the heterozygote state and their iron loading status was comparable to the matched controls without such defects, it would appear that the accumulation of excess iron in HbH disease is more likely a result of increase dietary absorption secondary to ineffective erythropoiesis.


Congenital dyserythropoietic anemias (CDA) are genetic disorders characterized by anemia and ineffective erythropoiesis. Three main types of CDA have been distinguished: CDA I, CDAII and CDA III, whose loci have been already mapped. After the identification of the locus for CDA II, also known as HEMPAS (hereditary erythroblast multinucularity with positive acidified serum test), on the long arm of chromosome 20 (20q11.2) we have analyzed by a mutational search seven candidate genes in a large series of CDA II patients. In particular, the following genes have been investigated: integrin beta 4 binding protein, ribophorin II, ubiquitin protein ligase ITCH, mannosyl-oligosaccharide alpha-1,2-mannosidase like protein, erythrocyte protein band 4.1 like protein, zinc finger protein PLAGL2, and finally novel zinc finger protein. None of them resulted as the causative gene but several protein variants and DNA polymorphisms have been identified. These data exclude the role of the above mentioned genes in causing CDA II and add further information in the process of cloning the CDA II gene.

suggest that the presence of the IVS5+1 G→A and H63D mutations should be considered when investigating iron overload in Vietnamese patients and those of mixed origin as co-inheritance of both mutations is likely to be a risk factor for iron overload.


Acute intermittent porphyria (AIP) is a very rare autosomal dominant disorder with low penetrance. Mutations in the gene of the porphobilinogen deaminase (PBG-D), also called hydroxymethylbilane synthase (HMBS), cause a partial deficiency of this enzyme of the heme biosynthetic pathway. Overstimulation of heme biosynthesis causes clinical symptoms. Because of the variability of the symptoms, diagnosis is often delayed. Using two approaches for genetic analysis, first in a stepwise manner, then sequencing extensive parts of the gene, the screening of the DNA of 20 unrelated individuals revealed 20 different mutations, 11 of which had not been reported previously. The novel mutations affected intron 1 (33 + 2 T→C), exon 5 (181 G→C), intron 6 (267-61 del 8 bp), intron 7 (345-1 G→C), intron 9 (498 + 15 G→T and 499-13 [Delta]-14 bp indel TGA), intron 13 (825 + 1 G→C and 825 + 2 T→C), exon 15 (962 G-A, 1067 del A and 1067-1068 ins 5 bp). The other nine mutations detected affected intron 14, exons 6, 7, 8, 9, 10 (3 x) and 12. In the majority of AIP patients, the genotype does not predict phenotypic expression. Since the sudden manifestation of the disease maybe prevented by early diagnosis, identification of AIP gene carriers is the best preventive measure. This was performed in five families, revealing 10 additional AIP gene carriers.


Objectives: To investigate the role of human papillomavirus (HPV) in the development of cervical neoplasia in women with no previous cervical cytological abnormalities; whether the presence of virus DNA predicts development of squamous intraepithelial lesion; and whether the risk of incident squamous intraepithelial lesions differs with repeated detection of the same HPV type versus repeated detection of different types. Design: Population based prospective cohort study. Setting: General population in Copenhagen, Denmark. Participants: 10 758 women aged 20-29 years followed up for development of cervical cytological abnormalities; 370 incident cases were detected (40 with atypical squamous cells of undetermined significance, 165 with low grade squamous intraepithelial lesions, 165 with high grade squamous intraepithelial lesions). Main outcome measures: Results of cervical smear tests and cervical swabs at enrolment and at the second examination about two years later. Results: Compared with women who were negative for human papillomavirus at enrolment, those with positive results had a significantly increased risk
at follow up of having atypical cells (odds ratio 3.2, 95% confidence interval 1.3 to 7.9), low grade lesions (7.5, 4.8 to 11.7), or high grade lesions (25.8, 15.3 to 43.6). Similarly, women who were positive for HPV at the second examination had a strongly increased risk of low (34.3, 17.6 to 67.0) and high grade lesions (60.7, 25.5 to 144.0). For high grade lesions the risk was strongly increased if the same virus type was present at both examinations (813.0, 168.2 to 3229.2).

Conclusions: Infection with human papillomavirus precedes the development of low and high grade squamous intraepithelial lesions. For high grade lesions the risk is greatest in women positive for the same type of HPV on repeated testing. What is already known on this topic Persistence of infection with human papillomavirus (HPV) is thought to have a role in the development of cervical neoplasia Previous studies have included only a few cases of high grade squamous intraepithelial lesions, and few have randomly sampled women from the general population What this study adds In women aged 20-29, HPV infection preceded the development of high grade lesions Persistent HPV infection with a specific HPV type was an indicator of incident high grade lesions among young women in the general population The association between persistence and high grade cervical lesions was more pronounced among women aged over 25

Bone (23)


http://www.sciencedirect.com/science/article/B6T4Y-3W37W6M-4/2/35d12643ceb655071e2470742f64d6ca

Parathyroid hormone (PTH) is a potent stimulator of osteoblastic cell function in vitro and bone resorption and formation in vivo; however, the details of the molecular mechanism(s) responsible for PTH action and the regulation of gene expression in response to PTH remain unknown. In this study, we employed an mRNA differential display (DRD) approach to examine the initial events in gene expression in human osteoblast-like SáoS-2/B10 cells exposed to 10-7 mol/L bPTH(1-34). This approach identified several differentially regulated mRNA species, including a novel paired-class homeobox protein, osteoblast-specific factor-2 (OSF-2), and a unique clone with no known sequence homology (clone G18). G18 is a previously unidentified human gene, expressed in a wide variety of human tissues, including heart, brain, placenta, skeletal muscle, and kidney, and is regulated by PTH in osteoblastic cells in vitro. This mRNA appears to be the product of a single gene, which is alternatively spliced to produce multiple transcript sizes observed in several tissues, except bone and bone-derived cells, in which a single predominant ~1.8 kb transcript is observed. Our study has identified several genes that have expression altered significantly by treatment with bPTH(1-34), and which may provide insight into the immediate effects of PTH on osteoblast-like cells and ultimately on the mechanism of action and bioactivity of PTH.


http://www.sciencedirect.com/science/article/B6T4Y-3RJPBBB-1F/2/d5bed5215a5044db78c84513fc842ba4
Mutations in the arg201 codon of the [alpha]s G protein subunit have been associated with a variety of disorders, but analysis of such mutations has been complicated by their mosaic presentation. To overcome the problems associated with the analysis of genomic mutations that may be present in low and variable yield throughout the body, a polymerase chain reaction (PCR)-based technique has been developed that allows the selective amplification of products from the mutant allele. This technique uses site-directed mutagenesis to generate a PCR product from the normal allele that is susceptible to restriction endonuclease digestion, whereas that from the mutant allele is resistant to digestion. Consecutive and repeated cycles of amplification and digestion allow selective enrichment of the product from the mutant allele. The technique has been applied to the analysis of patients with fibrous dysplasia of bone, where the consequence of G[alpha]s mutations may vary from monostotic to polyostotic lesions, and has been performed with DNA isolated from either bone biopsy specimens or peripheral blood leukocytes. In addition to the previously described arg -> his and arg -> cys substitutions, the analyses have detected a novel arg -> ser substitution in one of the patients. This patient presented with a panostotic disease and may represent a unique subgroup of fibrous dysplasia.


Bone loss is observed after exposure to weightlessness in both astronauts and inflight animals. Histological and biochemical studies on rats have shown a decrease in bone formation, probably as a result of altered osteoblast function. To investigate whether microgravity alters osteoblast differentiation in vitro, the human osteosarcoma cell line MG-63 was used as a model. MG-63 cells can be induced to differentiate by treating the cells with 1,25(OH)2D3 (10-7 mol/L) and transforming growth factor-beta 2 (TGF[beta]2) (10 ng/mL). The message level of differentiation-related genes was quantitated via competitive reverse transcription-polymerase chain reaction (RT-PCR), both in untreated and hormone-treated cells cultured under microgravity for 9 days aboard the unmanned Foton 10 spaceflight, and compared to ground and inflight unit-gravity cultures. At microgravity, gene expression for collagen I[alpha]1 following treatment was reduced to 51% of unit-gravity levels (p p p < 0.02). In conclusion, microgravity reduces the differentiation of osteoblastic MG-63 cells in response to systemic hormones and growth factors.


Bisphosphonates (BPs) are widely used in the treatment of a variety of bone-related diseases, particularly where the bone turnover is skewed in favor of osteolysis. The mechanisms by which BPs reduce bone resorption directly acting on osteoclasts are now largely clarified even at molecular level. Researches concerning the BP's effects on osteoblast have instead shown variable results. Many in vitro studies have reported positive effects on osteoblasts proliferation and mineralization for several BPs; however, the observed effects differ, depending on the variety of different model system that has been used. Objectives. We have investigated if neridronate, an aminobisphosphonate suitable for pulsatory parenteral administration, could have an effect on human osteoblastic proliferation and differentiation in vitro. Methods. We have investigated whether prolonged addition of neridronate (from 10-3 to 10-11 M) to different human osteoblasts cultures, obtained from 14 different bone specimens, could affect the cells number, the
endogenous cellular alkaline phosphatase (ALKP) activity, and the formation of mineralized nodules. Results. Our results show that neridronate does not negatively affect in vitro the viability, proliferation, and cellular activity of normal human osteoblasts even after a long period addition of the drug (20 days) at concentrations equal or lower than 10-5 mol/l (therapeutic dose). In addition, neridronate seems to enhance the differentiation of cultured osteoblasts in mature bone-forming cells. A maximum increase of alkaline phosphatase activity (+50% after 10 days; P P -8 M. Conclusions. These results encourage the use of neridronate in long-term therapy of demineralizing metabolic bone disorders.


http://www.sciencedirect.com/science/article/B6T4Y-42YF54G-5/2/fd81755726a256e87db3b8c8379af770

Bone morphogenetic protein (BMP)-2, a member of the transforming growth factor-[beta] (TGF-[beta]) superfamily, is able to induce osteoblastic differentiation of C2C12 cells. Both Smad and mitogen-activated protein kinase (MAPK) pathways are essential components of the TGF-[beta] superfamily signaling machinery. Although Smads have been demonstrated to participate in the BMP-2-induced osteoblastic differentiation of C2C12 cells, the role of MAPK has not been addressed. This report shows that BMP-2 activates ERK and p38, but not JNK, in C2C12 cells. Pretreatment of cells with the p38 inhibitor, SB203580, dramatically reduced BMP-2-induced expression of the osteoblast markers alkaline phosphatase (ALP) and osteocalcin (OC). Nevertheless, overexpression of MKK3, a protein kinase that phosphorylates and activates p38, failed to induce ALP or OC expression in the absence of BMP-2, indicating that p38 activation is necessary but not sufficient for the acquisition of the osteoblast phenotype by these cells. Although ALP induction was increased slightly in the presence of PD-98059, a selective inhibitor of the ERK cascade, this compound significantly inhibited both steady-state and BMP-2-induced OC RNA levels. Our results indicate that p38 and ERK cascades play a crucial role in the osteoblast differentiation of C2C12 cells mediated by BMP-2.


http://www.sciencedirect.com/science/article/B6T4Y-4CN2K4G-18/2/5adb5760e825648740f54b0067181eac

We have conducted a genome-wide scan on a pedigree containing 372 adult members, of whom 49 have PDB. In the present study, we report linkage of a large pedigree to the PDB3 region on chromosome 5q35-qter with a peak multipoint LOD score of 6.77. Sequestosome 1 (SQSTM/p62) has been identified as the causative PDB gene in this region. Six sequestosome 1 mutations have been described to date. Four mutations have been identified in exon 8, 1210delT and 1215delC both resulting in premature stop codon at amino acid 394, 1215C to T (P392L), 1224insT (E396X), one mutation in exon 7, 1200C to T (P387L) and a G to A splice junction mutation at IVS7+1. These mutations cluster in the C terminus of the protein and are predicted to disrupt the ubiquitin binding properties of sequestosome 1. Sequence analysis of the gene encoding sequestosome 1 revealed a single base pair deletion (1215delC) segregating with the majority of affected members in the pedigree. This deletion introduces a stop codon at position 394, resulting in premature termination of the protein (L394X) and loss of the ubiquitin-associated binding domain. Screening of affected members from 10 further PDB families identified the previously reported P392L mutation in one family. No SQSTM1/p62 coding mutations were found
in the remaining 9 families or in 113 age-matched controls.


Osteoarthritis (OA) is a common age-related joint disease resulting in progressive degenerative damage to articular cartilage. The etiology of primary OA has not yet been determined. However, there is evidence supporting the hypothesis that primary OA is a disease affecting bone remodeling in addition to articular cartilage. In this study, we have used cDNA microarray analysis to compare gene expression in bone between normal (CTL) and OA individuals. Trabecular bone was sampled from the intertrochanteric region of the proximal femur, a site distal to the diseased hip joint. Total RNA was extracted from three pairs of age- and sex-matched CTL and OA bone samples, reverse-transcribed and radioactively labeled to generate cDNA probes, before hybridization with the Research Genetics GF211 human gene microarray filter. The CTL and OA samples were found to have similar levels of gene expression for more than 4000 known human genes. However, forty-one genes were identified that were differentially expressed, twofold or more, between all three CTL-OA sample pairs. Using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis, three genes, fms-like tyrosine kinase 1 (FLT1), plexin B1 (PLXNB1), and small inducible cytokine A2 (SCYA2), were confirmed to be consistently expressed at lower levels in OA, in a majority of twenty age- and sex-matched CTL-OA bone sample pairs tested. FLT1, PLXNB1, and SCYA2 have known or potential roles in angiogenesis and bone remodeling. Down-regulation of these genes is consistent with a role for bone in the pathogenesis of OA.


http://www.sciencedirect.com/science/article/B6T4Y-3TGVHGX-3/2/babc8a8aeafad20d9ed2295974fc8d0c

We determined the effect of basic fibroblast growth factor (bFGF) on osteoclast-like cell (OCL) formation in bone marrow cultures using C57BL/6 mice. Cells were cultured for 7 days with or without bFGF at various concentrations or 10-8 mol/L 1,25(OH)2 vitamin D3 [1,25(OH)2D3]. bFGF dose-dependently increased OCL formation per well (10-10 mol/L = 40 +/- 2; 10-9 mol/L = 146 +/- 13; 10-8 mol/L = 156 +/- 12) compared with control (-9 and 10-8 mol/L were similar to that of 10-8 mol/L 1,25(OH)2D3 (154 +/- 11 per well). OCLs formed by bFGF were multinuclear, tartrate-resistant acid phosphatase (TRAP)-positive, expressed calcitonin receptors, and formed characteristic resorption pits. We also determined whether bFGF enhanced OCL formation during the early proliferative or late differentiating phases of the cultures. When bFGF (10-8 mol/L) was added only on days 1-2 or days 3-4 of 6 day cultures, there was a significant increase in OCL formation. In contrast, when bFGF was added only on days 5-6 few OCLs formed. Addition of bFGF at days 1-6 or days 1-2 and days 5-6 caused similar increases in OCL formation, which were greater than OCL formation induced by treatment for days 1-2 or days 1-4. We examined the production of prostaglandin E2 (PGE2) in the cultures because bFGF is a potent stimulator of PGE2 synthesis in bone, and PGE2 stimulates OCL formation. bFGF treatment significantly increased PGE2 levels in 7 day cultures (controls = 1.4 +/- 0.1 nmol/L, 10-8 mol/L bFGF = 132.5 +/- 0.7 nmol/L). In addition, treatment of marrow cultures with the prostaglandin synthesis inhibitors, indomethacin or NS-398 (both at 10-6 mol/L), completely blocked bFGF-induced OCL formation. We conclude that bFGF stimulates OCL formation in C57BL/6 bone marrow cultures
by mechanisms that require prostaglandin synthesis. This pathway is likely to be one mechanism by which bFGF stimulates resorption.


Prostaglandin (PG) E2 displays physiological and pharmacological action in various tissues including bone. It increases intracellular Ca, and stimulates or inhibits CAMP production through the PGE receptor subtypes EP1 EP2, and EP3 respectively. These receptor subtypes have been recently cloned. In the present study, we investigate the expression of these receptor subtypes in bone tissue. RT-PCR revealed that EP1 EP2, and EP3 were expressed in rat calvariae and that osteoblastic cells (MC3T3-E1) expressed EP1 and EP2. In situ hybridization analysis using cryosection of neonatal calvariae revealed that EP2 was expressed by osteoblasts and cells not in contact with bone, probably including preosteoblasts. EP2 expression was observed at an early stage in calvarial development, at 14 days prenatal. EP2 expression was also observed at day 3 in rat bone marrow cell culture in which bone-like mineralized nodules are formed at day 8. It has been established that PGE2 response accompanying cAMP production is one of the characteristics of osteoblasts. The present results indicate that this phenotype appears at an early stage of osteoblastic differentiation and bone development.


http://www.sciencedirect.com/science/article/B6T4Y-40J1DCN-9/2/739b94b3b012937f8f388ab5ca422140

Fibrillin-containing microfibrils are structural components of extracellular matrices of a diverse range of tissues, including bone. Their importance in bone biology is illustrated by the skeletal abnormalities manifest in the congenital disorder, Marfan syndrome, which results from mutations in the fibrillin-1 gene. We investigated the expression of fibrillins and other microfibril-associated proteins in human bone and bone-derived osteoblasts. Analysis of RNA extracted from cancellous bone showed expression of mRNAs encoding fibrillin-1 and -2, MAGP-1 and -2, LTBP-2, and MP78/70 (Big-h3). In demineralized normal mature bone, fibrillin-1 was immunolocalized to fibrils within the bone matrix and pericellularly to cells lining the endosteal surfaces of trabecular bone, some osteocytes, and cells associated with blood vessels. LTBP-2 was also identified at the endosteal surface and within the bone matrix in a lamellar fashion. In addition, primary osteoblast-like cells cultured from human trabecular bone (obtained from patients at joint replacement surgery) were found to express abundant mRNA for fibrillins and associated glycoproteins. Moreover, using western blot analysis, fibrillin-1 protein was shown to be secreted into the medium and to be deposited into the cell layer. Immunofluorescence staining of the cell layer visualized fibrillin-1 in the matrix as a three-dimensional network of fine filaments. Expression of fibrillin-1 by osteoblast-like cells was constitutive, and a number of skeletally active agents had little effect on mRNA or protein levels. These results show that human osteoblasts from mature bone express fibrillins and other microfibril-associated proteins, and suggest a role for these molecules in adult human bone.

http://www.sciencedirect.com/science/article/B6T4Y-47RBF85-2/2/db8ada0156c3e349ae62cfc63a408c

Transforming growth factor (TGF)-beta1 is the most abundant growth factor in human bone. It is produced by osteoblasts and inhibits osteoclast proliferation and activity and stimulates proliferation and differentiation of preosteoblasts. Several polymorphisms have been described in the TGF-beta1 gene. Previously, we and others have found associations between some of these polymorphisms and bone mass. We therefore wanted to examine if these polymorphisms are also predictors of osteoporotic fractures. The polymorphisms G-1639-A, C-1348-T, C-765insC, T29-C, G74-C, 713-8delC, C788-T, and T816-20-C were examined using RFLP and sequencing in 296 osteoporotic patients with vertebral fractures and 330 normal individuals. Bone mineral density (BMD) was examined at the lumbar spine and at the femoral neck by DXA. Genotype distributions were in H-W equilibrium. Linkage disequilibrium was found between the polymorphisms. The T816-20-C genotypes were distributed differently among osteoporotic patients and normal controls. The TT genotype was less common in individuals with osteoporotic fractures (T816-20-C) at the lumbar spine, 0.960 +/- 0.173 g/cm2 compared with individuals with the TC or CC genotypes: 0.849 +/- 0.181 g/cm2 and 0.876 +/- 0.179 g/cm2, respectively (P -1348-T) had higher bone mass at the femoral neck: 0.743 +/- 0.134 g/cm2 compared with 0.703 +/- 0.119 g/cm2 in individuals with TC or CC genotypes (P 29-C) had higher bone mass at the femoral neck, 0.735 +/- 0.128 g/cm2 compared with 0.703 +/- 0.120 g/cm2 in individuals with TC or TT genotypes (P 816-20-C polymorphism is less common in patients with osteoporotic fractures and is associated with higher bone mass both at the lumbar spine and at the hip. The C-1348-T and T29-C polymorphisms were distributed similarly in osteoporotic patients and normal controls, however, the rare genotypes were associated with higher bone mass at the hip.

Langdahl, B. L., J. Y. Knudsen, et al. (1997). "A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women." Bone 20(3): 289.

http://www.sciencedirect.com/science/article/B6T4Y-3RJ9105-J/2/db18252d6b597aa01a0abd9ac7da01b

Bone mass is partly genetically determined. The genes involved are, however, still largely unknown. Transforming growth factor-beta 1 (TGF-beta1) is considered a putative regulator of osteoclastic-osteoblastic interaction (coupling). The aim of the present study was therefore to examine whether possible variants of the TGF-beta1 gene are related to bone mass and osteoporosis. We examined 161 osteoporotic women (at least one low energy spinal fracture) and 131 normal women. We investigated sequence variations in the TGF-beta1 gene using the single-stranded conformation polymorphism (SSCP) technique combined with DNA sequencing. Seven patients were heterozygous for a cytosine to thymidine base substitution at position 76 in exon 5 (C788-T) (corresponding to position 788 in the TGF-beta1 cDNA), resulting in a threonine to isoleucine amino acid shift at position 263 in the TGF-beta1 propeptide (Thr263-Ile). Ten other patients had a one base deletion in the intron sequence 8 bases prior to exon 5 (713-8delC), which could influence splicing. Five normal women exhibited the C788-T sequence variant, and two the 713-8delC. The prevalence of 713-8delC was significantly higher in the osteoporotic group ([chi]2 = 4.02, p p z score of the lumbar spine below -1 were examined separately, we found increased serum levels of bone alkaline phosphatase (p p p < 0.05). The sequence variation, 713-8delC, in the TGF-beta1 gene is more frequent in patients with osteoporosis compared to normal controls. The 713-8delC variant seems to be associated with
very low bone mass in osteoporotic women with low bone mass and increased bone turnover in both osteoporotic and normal women.


http://www.sciencedirect.com/science/article/B6T4Y-3WS62VT-4/2/b0e7fa04066e7819ac6b8461e66e8bb6

We studied differences in ectopic osteointduction in eight mouse inbred strains and an outbred strain. Antigen-extracted autolyzed rat bone gelatin was implanted under hind limb muscle fascia of 12-week-old males, and new bone formation was morphologically assessed on serial sections. Four weeks after implantation, less than half of the implants from CBA/J, A/J, BALB/cJ, and C3Hf/Bu mice showed induction of only cartilage. New cartilage was observed in all, and bone and bone marrow in 80% of the implants from AKR/J, C57BL/6J, DBA/2J, and RFM/Rij mice. Volume of the newly formed tissue ranged from 1.3% of the old matrix in A/J strain to 74.6% in DBA/2J strain. Outbred CD1 mice showed only weak cartilage induction. The "good" responders differed among themselves in the volume and type of newly induced tissue: DBA/2J, RFM/Rij, and AKR/J mice had a similar ratio of new bone and cartilage and abundant bone marrow, whereas the predominant newly induced tissue in C57BL/6J mice was cartilage. The pattern of the expression of BMP-2, -4, and -7, alkaline phosphatase, osteocalcin, interferon-\[gamma\], and granulocyte-macrophage colony-stimulating factor, measured by reverse transcriptase polymerase chain reaction, did not correlate with the type and the quantity of the newly induced tissue. Our results show that adult mice of inbred strains differ not only in the peak bone mass and morphology, but also ability to form new bone after an osteoinductive stimulus. Ectopic osteoinduction may be a useful in vivo model to investigate genetic determinants of endochondral osteogenesis, especially its immunological component.

Nelson, A. E., R. S. Mason, et al. (1998). "Tumor expression studies indicate that HEM-1 is unlikely to be the active factor in oncogenic osteomalacia." Bone 23(6): 549.

http://www.sciencedirect.com/science/article/B6T4Y-3V5TMGJ-9/2/4a74de1eebd1f2a4623cfa0c2c9f2793

HEM-1 was isolated as a putative factor responsible for oncogenic osteomalacia by Kumar et al. (Proc Assoc Am Phys 107:296-305; 1995). The cDNA was identified on the basis of PTH-like immunoreactivity; however, no studies have been reported of the expression of HEM-1 mRNA in oncogenic osteomalacia tumors. In this study, expression of HEM-1 mRNA was investigated in two oncogenic osteomalacia tumors and in a series of normal tissues. An HEM-1 PCR product was amplified from a cDNA library from one of the tumors, with six base changes identified, as compared with the published sequence. No expression was detected, however, in the oncogenic osteomalacia tumors either by Northern blot analysis or by reverse transcriptase PCR. This indicates that, although a region of HEM-1 sequence is present in the tumor cell cDNA library, any HEM-1 expression must be at very low levels. It is unlikely, therefore, that the HEM-1 product is the active factor responsible for oncogenic osteomalacia. In the normal tissues examined, human placenta, fibroblasts, parathyroid gland, liver, fetal bone, and rat kidney cortex, HEM-1 mRNA was not detected, suggesting that it does not have a physiological role in these tissues.

The pathogenesis of osteoporosis involves both genetic and environmental factors. On the basis of linkage data suggesting gene effects on bone density at chromosome 14q and data locating the BMP4 gene to 14q, we performed a positional candidate study to examine a possible association of BMP4 gene polymorphisms, hip bone density ($n = 1012$) and fracture rates ($n = 1232$) in postmenopausal women (mean age 75). On genotype analysis of the three selected single nucleotide polymorphisms (SNP), the 6007C > T polymorphism was associated with total and intertrochanteric hip BMD and BMD was lower in the 32% of subjects homozygous for the C allele. This polymorphism codes for a nonsynonymous amino acid change with the T allele coding for valine, while the C allele codes for alanine. The difference in BMD was 3.1% (TT vs. CC) and 2.3% (CT versus CC) for the total hip ($P = 0.023$), and 3.7% (TT vs. CC) and 2.8% (CT versus CC) for the intertrochanter site ($P = 0.012$). Haplotype analysis demonstrated 6 haplotypes of frequency greater than 2%. A major haplotype defined by G-C-T alleles in SNPs -5826G > A, 3564C > T and 6007C > T respectively, showed association with high bone mass. No SNP showed association with fracture rates. We conclude that a polymorphism found in the BMP4 gene, affecting amino acid sequence, is associated with hip bone density in postmenopausal women, presumably via regulation of anabolic effects on the skeleton.


Tartrate-resistant acid phosphatase (TRAP) is expressed at high levels in osteoclasts and may play an important role in the bone resorptive process. However, factors regulating human TRAP gene expression have not been clearly defined. Therefore, we isolated a genomic clone (CL-9) for TRAP containing a 14-kb insert. A restriction map was generated for this insert, and a 2.6-kb Apal fragment containing the 5'-flanking region was subcloned. Sequence analysis of this fragment revealed the presence of candidate transcription factor-binding sequences for H-APF-1, SP1, GATA2, and the c-Myc proto-oncogene. PCR analysis of RNA isolated from human osteoclastomas and pagetic bone revealed a 276-bp intron at -1 by to -276 bp relative to the ATG and a transcript originating from this intron. Rapid amplification of the 5' end of the human TRAP mRNA by PCR indicated the presence of a 93-bp untranslated region 5' from the intron. Promoter activity was detected in the DNA fragment from +1 bp to -1903 by relative to the ATG initiation codon, which drove the transient expression of a luciferase reporter gene when transfected into HRE H9 rabbit endometrial cells. Comparison of the human TRAP 5'-flanking region with mouse TRAP and uteroferrin revealed 41% and 47% homology, respectively. This suggests that regulation of human TRAP gene expression may differ from that for the murine TRAP gene.


Silicon deficiency in animals leads to bone defects. This element may therefore play an important role in bone metabolism. Silicon is absorbed from the diet as orthosilicic acid and concentrations in plasma are 5-20 [mu]M. The in vitro effects of orthosilicic acid (0-50 [mu]M) on collagen type 1
synthesis was investigated using the human osteosarcoma cell line (MG-63), primary osteoblast-like cells derived from human bone marrow stromal cells, and an immortalized human early osteoblastic cell line (HCC1). Collagen type 1 mRNA expression and prolyl hydroxylase activity were also determined in the MG-63 cells. Alkaline phosphatase and osteocalcin (osteoblastic differentiation) were assessed both at the protein and the mRNA level in MG-63 cells treated with orthosilicic acid. Collagen type 1 synthesis increased in all treated cells at orthosilicic acid concentrations of 10 and 20 \( \mu \text{M} \), although the effects were more marked in the clonal cell lines (MG-63, HCC1 1.75- and 1.8-fold, respectively, \( P = 0.004 \)). The effect of orthosilicic acid was abolished in the presence of prolyl hydroxylase inhibitors. No change in collagen type 1 mRNA level was seen in treated MG-63 cells. Alkaline phosphatase activity and osteocalcin were significantly increased (1.5, 1.2-fold at concentrations of 10 and 20 \( \mu \text{M} \), respectively, \( P < 0.05 \)). Gene expression of alkaline phosphatase and osteocalcin also increased significantly following treatment. In conclusion, orthosilicic acid at physiological concentrations stimulates collagen type 1 synthesis in human osteoblast-like cells and enhances osteoblastic differentiation.


Skeletal unloading results in an inhibition of bone formation associated with a decrease in osteoblast number, impaired mineralization of bone, and altered proliferation and differentiation of osteoprogenitor cells. Although such changes are likely to be mediated by multiple factors, resistance to the growth-promoting action of insulin-like growth factor I (IGF-I) has been hypothesized to play an important role. To determine whether skeletal unloading induces resistance to IGF-I on bone formation, we examined the response of unloaded (hindlimb elevation) and normally loaded tibia and femur to IGF-I administration. To eliminate the variable of endogenous growth hormone production and secretion during exogenous IGF-I administration, we used growth hormone-deficient dwarf rats (dw-4). The rats were given IGF-I (2.5 mg/kg/day) or vehicle during 7 and 14 days of unloading or normal loading. This significantly increased the serum level of IGF-I in both the normally loaded and unloaded rats. Unloading did not affect the serum level of IGF-I in the vehicle-treated rats. IGF-I markedly increased periosteal bone formation at the tibiofibular junction of normally loaded rats. Unloading decreased bone formation in the vehicle-treated rats, and blocked the ability of IGF-I to increase bone formation. On the other hand, IGF-I increased periosteal bone formation at the midpoint of the humerus (normally loaded in this model) in both hindlimb-elevated and normally loaded rats. IGF-I significantly increased osteogenic colony number, total ALP activity, and total mineralization in bone marrow osteoprogenitor (BMOp) cells of normally loaded rats. Unloading reduced these parameters in the vehicle-treated rats, and blocked the stimulation by IGF-I. Furthermore, IGF-I administration (10 ng/ml) in vitro significantly increased cell proliferation of the BMOp cells isolated from normally loaded bone, but not that of cells from unloaded bone. These results indicate that skeletal unloading induces resistance to IGF-I on bone formation.


Interleukin-6 (IL-6) has been attributed to induction of osteoclastogenic-precursor cell proliferation and maturation. Estrogens suppress IL-6 production in stromal/osteoblastic cells in vitro.
Conversely, estrogen withdrawal is associated with increased IL-6 production. IL-6 is therefore thought to be an important mediator of the increased bone resorption after menopause. However, evidence supporting a rise in the expression of IL-6 or the IL-6 receptor in human bone tissue with menopause is still lacking. To address this question, we established a 5'-nuclease assay to quantitate the expression of human IL-6 and the gp80 subunit of the IL-6 receptor in human bone samples. The number of mRNA copies was normalized to the number of copies of beta actin mRNA. Osteocalcin expression served as an independent control. The study population consisted of 169 women (mean age 52.4 +/- 11.6 years) who underwent surgery for early breast cancer. Serum IL-6 was measured by enzyme-linked immunosorbent assay, serum crosslaps as a marker of bone resorption were measured by electrochemiluminescent assay, and serum osteocalcin was measured by chemoluminescence assays. RNA expression of osteocalcin in bone tissue from early postmenopausal women was higher compared with premenopausal women. Local expression was positively associated with circulating osteocalcin and crosslaps concentrations. Postmenopausal women also had higher circulating IL-6 concentrations. In contrast, bone samples from postmenopausal women lacked an increased expression of either IL-6 or gp80 compared with bone samples from premenopausal women. In conclusion, we failed to detect local increases in IL-6 or IL-6 receptor expression in human bone tissue with menopause. If direct changes in the IL-6 system in bone tissue are involved in postmenopausal bone loss, these changes appear to be below the detection limit of our assay system.


http://www.sciencedirect.com/science/article/B6T4Y-43MDDG9-W/2/39f5e529059e5c43bb9cc48b227d0920

Ferritin, a metal-binding protein responsible for maintaining the bioavailability of iron, has been demonstrated in cells of the osteoblastic lineage. Messenger RNAs encoding the light and heavy chain subunits of ferritin were detected in ROS 17/2.8, ROS 25/1, and UMR106 rat osteosarcoma cell lines, in fetal rat calvaria, and in primary cultures of rat calvarial osteoblast-like cells. In vivo, the expression of ferritin light-chain mRNA was observed in both active osteoblasts and in osteocytes. A 450-kD iron-binding protein was immunoprecipitated from ROS 17/2.8 cells by an antiferritin antiserum. This protein comigrated with native ferritin, and could be dissociated into subunits comigrating with ferritin light and heavy chains. Addition of extracellular Fe59-transferrin to cultures of ROS 17/2.8 cells resulted in the sequestration of the iron in intracellular ferritin. These observations demonstrate that cells of the osteoblastic lineage possess a functional ferritin-based iron uptake and storage system capable of regulating metal homeostasis in bone.


http://www.sciencedirect.com/science/article/B6T4Y-4447H0C-2/2/6a0d95cc6a20a2a02acb7b6df6e5e3fd4

Several members of the transforming growth factor-[beta] (TGF-[beta]) superfamily have been demonstrated to play regulatory roles in osteoblast differentiation and maturation, but the mechanisms by which they act on different cells at different developmental stages remain largely unknown. We studied the effects of TGF-[beta]1 and bone morphogenetic protein-2 (BMP-2) on the differentiation/maturation of osteoblasts using the murine cell lines MC3T3-E1 and C3H10T1/2. BMP-2 induced or enhanced the expression of the osteoblast differentiation markers alkaline phosphatase (ALP) and osteocalcin (OC) in both cells. In contrast, TGF-[beta]1 was not only unable to induce these markers, but it dramatically inhibited BMP-2-mediated OC gene
expression and ALP activity. In addition, TGF-β1 inhibited the ability of BMP-2 to induce MC3T3-E1 mineralization. TGF-β1 did not sensibly modify the increase of Osf2/Cbfa1 gene expression mediated by BMP-2, thus demonstrating that the inhibitory effect of TGF-β1 on osteoblast differentiation/maturation mediated by BMP-2 was independent of Osf2/Cbfa1 gene expression. Finally, it is shown that TGF-β1 does not affect BMP-2-induced Smad1 transcriptional activity in the mesenchymal pluripotent cells studied herein. Our data indicate that in vitro BMP-2 and TGF-β1 exert opposite effects on osteoblast differentiation and maturation.


http://www.sciencedirect.com/science/article/B6T4Y-4BX2KR5-26/2/d89bc1bf0f18f265200669f2b0517aaa

Osteogenesis imperfecta (OI) is a group of inherited disorders characterized by a predisposition to bone fracturing, and usually resulting from mutations in the genes encoding type I collagen. This report describes the molecular defects in a patient with type II OI and another with type III OI. These patients were demonstrated to possess point mutations resulting in glycine -> arginine substitutions within the triple helical domain of the [alpha]1(I) or [alpha]2(I) collagen polypeptide chain. The defect in the type II OI patient affected residue 211 of the [alpha]1(I) triple helical domain, and constitutes the most amino-terminal lethal glycine -> arginine substitution described to date. The substitution in the type III OI patient affected residue 427 of the [alpha]2(I) triple helical domain. Both defects were informative in that they identified the regions of the [alpha]1(I) and [alpha]2(I) collagen chains in which the phenotypes associated with glycine -> arginine substitutions undergo a transition between lethal and nonlethal forms, thereby allowing a more reliable prognosis of disease severity. The histological examination of bone from these patients revealed striking abnormalities in the quantity and organization of mineralized bone structures, compared with age-matched controls. Although the patients were differently classified, no major differences in the magnitude of bone architectural changes could be perceived, consistent with the presence of their defects near a common phenotypic transition. The results are compatible with there being a gradient in severity between OI types II and III, and that parameters external to the gene mutations might account for the survival differences in the 2 cases presented in this study.


Several lines of evidence suggest that vitamin K has nutritional and pharmacological effects against bone loss. To clarify effects of vitamin K on bone marrow cells, which contains progenitors of both osteoblasts and osteoclasts, we examined mouse bone marrow cell cultures in the presence of vitamin K1 (K1) and menatetrenone (MK4), a vitamin K2 with four isoprene units. Treatment with MK4 but not K1 inhibited the formation of adipocytes and stimulated alkaline phosphatase activity, an early differentiation marker of osteoblast. Although nuclear receptor PPAR[gamma]2 plays a pivotal role in adipogenesis, MK4 had no effects on the expression of PPAR[gamma]2 mRNA and PPAR[gamma]2-dependent transcriptional activity. MK4 inhibited the expression of osteoclast differentiation factor (ODF)/RANK ligand and the formation of osteoclast-like cells induced by 1,25-dihydroxyvitamin D3. These results suggest that MK4 specifically influences differentiation and functions of bone marrow cells to inhibit adipogenesis and
osteoclastogenesis. At the expense of adipogenesis, MK4 might stimulate osteoblastogenesis in bone marrow cells. Therefore, MK4 may favor bone metabolism to spare bone mass as a compound that modulates cellular differentiation and functions in bone marrow in addition to as a nutrient factor.

Br. J. Ophthalmol. (4)


http://bjo.bmjjournals.com/cgi/content/abstract/86/3/328

Background: Besides the three known genes (RHO, RDS/Peripherin, NRL) involved in autosomal dominant retinitis pigmentosa (adRP), a fourth gene, RP1, has been recently identified. Initial reports suggest that mutations in the RP1 gene are the second most frequent cause of adRP. The clinical findings were described in a family with adRP and a novel mutation in the RP1 gene. Method: Index patients from 15 independent families with adRP in which RHO mutations had been excluded in previous examinations were screened for mutations in the RP1 gene by means of direct DNA sequencing. Evaluation of the RP1 phenotype in patients included funduscoppy, kinetic perimetry, dark adapted final threshold test, standard electroretinography and, in one case, multifocal electroretinography. Results: One novel nonsense mutation (Lys778ter) in one of these 15 patients was detected. Cosegregation of the mutation with the disease phenotype could be established in the index patient's family. The phenotype comprises variable expression of clinical disease probably including one case of incomplete penetrance, a onset of symptoms beginning in adulthood, and evidence of regionally varying retinal function loss. Conclusion: The Lys778ter mutation localises inside the critical region harbouring all mutations described so far. The ophthalmic findings support previous observations that variation of disease expression appears as a typical feature of the RP1 phenotype.


http://bjo.bmjjournals.com/cgi/content/abstract/87/7/893

Aim: To characterise the phenotype and identify the underlying genetic defect in a family with deafness segregating with a North Carolina-like macular dystrophy (NCMD). Methods: Details of the family were obtained from the Moorfields Eye Hospital genetic clinic database and comprised eight affected, four unaffected members, and two spouses. Pedigree data were collated and leucocyte DNA extracted from venous blood. Positional candidate gene and genetic linkage strategies utilising polymerase chain reaction (PCR) based microsatellite marker genotyping were performed to identify the disease locus. Results: The non-progressive ocular phenotype shared similarities with North Carolina macular dystrophy. Electro-oculography and full field electroretinography were normal. Progressive sensorineural deafness was also present in all affected individuals over the age of 20 years. Hearing was normal in all unaffected relatives. Haplotype analysis indicated that this family is unrelated to previously reported families with NCMD. Genotyping excluded linkage to the MCDR1 locus and suggested a potential novel
disease locus on chromosome 14q (Z=2.92 at \(\theta=0\) for marker D14S261). Conclusion: The combination of anomalies segregating in this family represents a novel phenotype. This molecular analysis indicates the disease is genetically distinct from NCMD.


http://bjo.bmjournals.com/cgi/content/abstract/86/7/767

Background: Glaucomatous neuropathy is a type of cell death by apoptosis. The p53 gene is one of the regulatory genes of apoptosis. Recently, p53 codon 72 polymorphism has been extensively studied to determine the risk factors responsible for many diseases. In the p53 gene, a single base change from G to C causes the alternation of amino acid residue 72 from arginine to proline. In this study the association between p53 codon 72 polymorphism and primary open angle glaucoma (POAG) patients was evaluated. Methods: 58 POAG patients and 59 healthy volunteers were enrolled in this study. Polymerase chain reaction based analysis was used to resolve the p53 codon 72 polymorphism. Results: There were significant differences in the distribution of the polymorphism between the control subjects and the POAG patients (p = 0.00782) The proline form of p53 gene codon 72 appears to be a significant risk factor in the development of POAG (odds ratio 2.389, 95% confidence interval: 1.14 to 5.01). Conclusions: Retinal ganglion cells die during POAG by apoptosis. The tumour suppressor protein, p53, is one of the primary regulators steps of apoptosis, and the results of our study are compatible with this concept.


http://bjo.bmjournals.com/cgi/content/abstract/88/6/752

Aim: To determine the disease causing gene defects in two patients with Meesmann's corneal dystrophy. Methods: Mutational analysis of domains 1A and 2B of the keratin 3 (K3) and keratin 12 (K12) genes from two patients with Meesmann's corneal dystrophy was performed by polymerase chain reaction amplification and direct sequencing. Results: Novel mutations of the K12 gene were identified in both patients. In one patient a heterozygous point mutation (429A[G]C = Arg135Ser) was found in the 1A domain of the K12 gene. This mutation was confirmed by restriction digestion. In the second patient a heterozygous 27 bp duplication was found inserted in the 2B domain at nucleotide position 1222 (1222ins27) of the K12 gene. This mutation was confirmed by gel electrophoresis. The mutations were not present in unaffected controls. Conclusion: Novel K12 mutations were linked to Meesmann's corneal dystrophy in two different patients. A missense mutation replacing a highly conserved arginine residue in the beginning of the helix initiation motif was found in one patient, and an insertion mutation, consisting of a duplication of 27 nucleotides, was found before the helix termination motif in the other.

http://brain.oupjournals.org/cgi/content/abstract/126/6/1293

The autosomal dominant cerebellar ataxias (ADCA) are a clinically, pathologically and genetically heterogeneous group of disorders. Ten responsible genes have been identified for spinocerebellar ataxia types SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12 and SCA17, and dentatorubral pallidoluysian atrophy (DRPLA). The mutation is caused by an expansion of a CAG, CTG or ATTCT repeat sequence of these genes. Six additional loci, SCA4, SCA5, SCA11, SCA13, SCA14 and SCA16 have also been mapped. The growing heterogeneity of the autosomal dominant forms of these diseases shows that the genetic aetiologies of at least 20% of ADCA have yet to be elucidated. We ascertained and clinically characterized a four-generation Chinese pedigree segregating an autosomal dominant phenotype for cerebellar ataxia. Direct mutation analysis, linkage analysis for all known SCA loci and a genome-wide linkage study were performed. Direct mutation analysis excluded SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and DRPLA, and genetic linkage analysis excluded SCA4, 5, 11, 13, 14 and 16. The genome-wide linkage study suggested linkage to a locus on chromosome 1p21-q23, with the highest two-point LOD score at D1S1167 (Zmax = 3.46 at \( \theta \) = 0.00). Multipoint analysis and haplotype reconstruction traced this novel SCA locus (SCA22) to a 43.7-cM interval flanked by D1S206 and D1S2878 (Zmax = 3.78 under four liability classes, and 2.67 using affected-only method). The age at onset ranged from 10 to 46 years. All affected members had gait ataxia with variable features of dysarthria and hyporeflexia. Head MRI showed homogeneous atrophy of the cerebellum without involvement of the brainstem. In six parent-child pairs, median onset occurred 10 years earlier in offspring than in their parents, suggesting anticipation. This family is distinct from other families with SCA and is characterized by a slowly progressive, pure cerebellar ataxia.


http://brain.oupjournals.org/cgi/content/abstract/127/12/2693

The pathophysiological mechanisms underlying the development of spasticity are not clear, but the excitability of the disynaptic reciprocal inhibitory pathway is affected in many patients with spasticity of different origin. Patients with genetically identified autosomal dominant pure spastic paraparesis (ADPSP) develop spasticity and paresis in the legs, but usually have no symptoms in the arms. Comparison of the spinal and supraspinal control of the legs and arms in these patients may therefore provide valuable information about the pathophysiology of spasticity. In the present study, we tested the hypothesis that one of the pathophysiological mechanisms of spasticity in these patients is abnormal corticospinal transmission and that this may lead to decreased reciprocal inhibition. Ten patients and 15 healthy age-matched control subjects were investigated. The patients were all spastic in the legs (with hyperactive tendon reflexes, increased muscle tone and Babinski sign), but had no neurological symptoms in the arms (except for one patient). Disynaptic reciprocal Ia inhibition of flexor carpi radialis (FCR) and soleus (SOL) motoneurons was measured (as the depression of the background FCR and SOL EMG activity and as the short latency inhibition of the FCR and SOL H-reflex evoked by radial and peroneal nerve stimulation). In addition, the latency of motor evoked potentials (MEPs) in the FCR muscle and the tibialis anterior (TA) muscle was measured. In the patients, the mean reciprocal inhibition was normal in the arms, while it was significantly decreased in the leg compared with the healthy subjects. In the patients, the average latency of MEPs in the FCR muscle was normal, while the latency to the MEP in TA muscle was significantly longer than that found in healthy subjects. Four patients, however, differed from the other patients by having significant reciprocal inhibition in the leg and a significantly shorter latency of TA MEPs than found in the other patients. The six patients without
reciprocal inhibition in the leg instead had significant short latency facilitation of the SOL H-reflex and a longer TA MEP latency than seen in the healthy subjects and in the four patients with retained reciprocal inhibition. These findings support the hypothesis that disynaptic reciprocal inhibition and short latency facilitation are involved in the development of spasticity and, furthermore, they suggest a positive correlation between impairment of corticospinal transmission and decrease of reciprocal inhibition/appearance of reciprocal facilitation.


Glutaric acidemia type 1 (GA-1) is an autosomal recessive disorder characterized by a deficiency of glutaryl-CoA dehydrogenase (GCDH) activity. GA-1 is often associated with an acute encephalopathy between 6 and 18 months of age that causes striatal damage resulting in a severe dystonic movement disorder. Ten autopsy cases have been previously described. Our goal is to understand the disorder better so that treatments can be designed. Therefore, we present the neuropathological features of six additional cases (8 months-40 years), all North American aboriginals with the identical homozygous mutation. This cohort displays similar pathological characteristics to those previously described. Four had macroencephaly. All had striatal atrophy with severe loss of medium-sized neurons. We present several novel findings. This natural time course study allows us to conclude that neuron loss occurs shortly after the encephalopathical crisis and does not progress. In addition, we demonstrate mild loss of large striatal neurons, spongiform changes restricted to brainstem white matter and a mild lymphocytic infiltrate in the early stages. Reverse transcriptase-PCR to detect the GCDH mRNA revealed normal and truncated transcripts similar to those in fibroblasts. All brain regions demonstrated markedly elevated concentrations of GA (3770-21 200 nmol/g protein) and 3-OH-GA (280-740 nmol/g protein), with no evidence of striatal specificity or age dependency. The role of organic acids as toxic agents and as osmolytes is discussed. The pathogenesis of selective neuronal loss cannot be explained on the basis of regional genetic and/or metabolic differences. A suitable animal model for GA-1 is needed.


Three genes commonly causing Charcot-Marie-Tooth disease (CMT) encode myelin-related proteins: peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ) and connexin 32 (Cx32). Demyelinating versus axonal phenotypes are major issues in CMT associated with mutations of these genes. We electrophysiologically, pathologically and genetically evaluated demyelinating and axonal features of 205 Japanese patients with PMP22 duplication, MPZ mutations or Cx32 mutations. PMP22 duplication caused mainly demyelinating phenotypes with slowed motor nerve conduction velocity (MCV) and demyelinating histopathology, while axonal features were variably present. Two distinctive phenotypic subgroups were present in patients with MPZ mutations: one showed preserved MCV and exclusively axonal pathological features, while the other was exclusively demyelinating. These axonal and demyelinating phenotypes were well concordant among siblings in individual families, and MPZ mutations did not overlap among these two subgroups, suggesting that the nature and position of the MPZ mutations mainly determine the axonal and demyelinating phenotypes. Patients with Cx32 mutations showed intermediate slowing of MCV, predominantly axonal features and relatively mild demyelinating
pathology. These axonal and demyelinating features were present concomitantly in individual patients to a variable extent. The relative severity of axonal and demyelinating features was not associated with particular Cx32 mutations. Median nerve MCV and overall histopathological phenotype changed little with disease advancement. Axonal features of diminished amplitudes of compound muscle action potentials (CMAPs), axonal loss, axonal sprouting and neuropathic muscle wasting all changed as disease advanced, especially in PMP22 duplication and Cx32 mutations. Median nerve MCVs were well maintained independently of age, disease duration and the severity of clinical and pathological abnormalities, confirming that median nerve MCV is an excellent marker for the genetically determined neuropathic phenotypes. Amplitude of CMAPs was correlated significantly with distal muscle strength in PMP22 duplication, MPZ mutations and Cx32 mutations, while MCV slowing was not, indicating that clinical weakness results from reduced numbers of functional large axons, not from demyelination. Thus, the three major myelin-related protein mutations induced varied degrees of axonal and demyelinating phenotypic features according to the specific gene mutation as well as the stage of disease advancement, while clinically evident muscle wasting was attributable to loss of functioning large axons.


http://brain.oupjournals.org/cgi/content/abstract/127/3/505

Symptoms of Huntington's disease may be caused by a toxic insult triggered by the mutant human huntingtin (Htt) protein itself, by a maladaptive protective mechanism initiated in response to an insult, or by a combination of these. We observed a protection from N-methyl-D-aspartate (NMDA) receptor-induced excitotoxicity in striata of symptomatic N171-82Q mice, a new transgenic model of Huntington's disease. The goal of this study was to determine if NMDA receptor-mediated signalling pathways are altered in these mice. Multiple proteins of NMDA receptor and dopamine D1 receptor pathways are being regulated in ways predictive of the protection we observe. Although examining NMDA receptor subunit proteins showed no change in NR1, NR2A, or NR2B in the striata of the symptomatic mice, we observed a decrease in phosphorylation of NR1 at Ser897, previously reported to decrease NMDA receptor current. The dopamine D1 receptor, responsible for protein kinase A activation and subsequent phosphorylation of Ser897 of NR1, also showed an age-related decrease. Other proteins regulated in this disease were associated with PSD-95-like scaffolding proteins of the NMDA receptor. Specifically, we observed a decrease in membrane-associated neuronal nitric oxide synthase (nNOS), a decrease in PSD-95-like proteins, which link nNOS to the NMDA receptor complex, and a decrease in citron, a protein associated with dendritic spine formation. From these data, we conclude that the N171-82Q mice seem to be regulating, in a protective direction, many of the known effector pathways of NMDA receptor-induced excitotoxicity. These regulations, although seemingly effective in decreasing neuronal death, may in fact be causing some of the symptoms associated with the disease.


http://brain.oupjournals.org/cgi/content/abstract/126/1/32

To date, two point mutations, G209A and G88C, have been reported in the coding region of the {alpha}-synuclein gene in autosomal dominant familial Parkinson's disease. When translated, these lead to the missense mutations Ala53Thr and Ala30Pro, respectively. Reduced mRNA expression of the G209A allele was reported recently in a Greek-American family. Here, we show that {alpha}-synuclein mRNA is normally expressed in blood cells and report the results of an
analysis of {alpha}-synuclein mRNA and protein expression in lymphoblastoid cell lines established from kindreds with the G209A and G88C mutations. mRNA expression was characterized using a TaqMan real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. We assessed five affected and three unaffected members of a German family with the G88C mutation and two affected members in different, unrelated Greek families with the G209A mutation. The ratio of wild-type to mutant {alpha}-synuclein allele expression ranged from 2.2 to 9.2 in the affected individuals with a severe clinical phenotype. The ratios of the expression levels of the wild-type to mutant alleles were only slightly decreased in mild cases and were less than 1.0 in two asymptomatic heterozygotes. Sequence analysis of the RT-PCR products showed only the presence of G in position 88 and G in position 209 in severely affected heterozygotes of the German and Greek families, respectively. High performance liquid chromatography/mass spectrometry demonstrated that, relative to wild-type {alpha}-synuclein, there is a reduction of Ala30Pro {alpha}-synuclein in lymphoblastoid cell lines originating from severely affected, but not mildly affected G88C/+ heterozygotes. Taken together, these data indicate that there is haploinsufficiency at the {alpha}-synuclein gene and that the ratio of expression of the wild-type to mutant alleles correlates with the severity of the clinical phenotype. Furthermore, these findings suggest that haploinsufficiency of {alpha}-synuclein mutations may contribute to disease progression in these forms of familial Parkinson's disease.


http://brain.oupjournals.org/cgi/content/abstract/127/11/2540

Autosomal recessive demyelinating Charcot-Marie-Tooth disease (CMT4), Dejerine-Sottas disease and congenital hypomyelinating neuropathy are variants of hereditary demyelinating neuropathy of infancy, a genetically heterogeneous group of disorders. To explore the spectrum of early-onset demyelinating neuropathies further, we studied the clinicopathological and genetic aspects of 20 patients born to unaffected parents. In 19 families out of 20, consanguinity between the parents or presence of an affected sib suggested autosomal recessive transmission. Screening of various genes known to be involved in CMT4 revealed six mutations of which five are novel. Four of these novel mutations occurred in the homozygous state and include: one in GDAP1, one in MTMR2, one in PRX and one in KIAA1985. One patient was heterozygous for a novel MTMR2 mutation and still another was homozygous for the founder mutation, R148X, in NDRG1. All patients tested negative for mutations in EGR2. Histopathological examination of nerve biopsy specimens showed a severe, chronic demyelinating neuropathy, with onion bulb formation, extensive demyelination of isolated fibres and axon loss. We did not discern a specific pattern of histopathology that could be correlated to mutations in a particular gene.


http://brain.oupjournals.org/cgi/content/abstract/126/8/1873

Interleukin (IL)-6 is a multifunctional cytokine with diverse actions and has been implicated in the pathophysiology of many neurological and inflammatory disorders. In this study, we investigated the role of IL-6 in pneumococcal meningitis. Cerebral infection in wild-type (WT) mice caused an increase in vascular permeability and intracranial pressure (ICP), which were significantly reduced in IL-6/-/- mice. In contrast, meningitis in IL-6/-/- mice was associated with a significant increase in CSF white blood cell count compared with infected WT mice, indicating an enhanced inflammatory response. Analysis of mRNA expression in the brain showed an increase in tumour necrosis factor (TNF)-{alpha}, IL-1{beta}, and macrophage inflammatory protein 2 (MIP-2) levels,
but decreased expression of granulocyte-macrophage colony-stimulating factor in infected IL-6-/- mice compared with infected WT controls. Similar results were obtained when rats challenged with pneumococci were systemically treated with neutralizing anti-IL-6 antibodies, resulting in an increased pleocytosis but at the same time a reduction of vascular permeability, brain oedema formation, and ICP, which was not accompanied by a downregulation of matrix metalloproteinases. Our data indicate that IL-6 plays an important anti-inflammatory role in bacterial meningitis by reducing leukocyte infiltration but contributes to the rise in intracranial pressure by increasing blood-brain barrier (BBB) permeability. These findings suggest that the migration of leukocytes across the BBB and the increase in vascular permeability are two independent processes during bacterial meningitis.


http://brain.oupjournals.org/cgi/content/abstract/126/4/814

The majority of cases with frontotemporal dementia (FTD) have no tau deposition in the brain, yet mutations in the tau gene lead to a similar clinical phenotype with insoluble tau depositing in neuropathological lesions. We report two tau gene mutations at positions +19 and +29, in the intronic sequences immediately following the stem loop structure in exon 10, which segregate with FTD. Exon-trapping experiments showed that these gene mutations alter the splicing out of exon 10 and produce an increase in tau isoforms with three microtubule binding domains (three repeat tau). Mutagenesis experiments demonstrated that the +19 mutation was responsible for the increase in three repeat tau, possibly by altering an intron silencer modulator sequence element found at this region of the gene. Microtubule binding experiments revealed a significant decrease in microtubule assembly with increasing amounts of three and decreasing amounts of four repeat tau. Brain autopsy was available in one case. Analysis of the type of soluble tau isoforms revealed an increase in three repeat tau and an absence of tau isoforms with exon 3 inserts. No insoluble tau was isolated in the tissue fractions, consistent with the absence of tau-positive histopathology. There was also an increase in tau degradation products suggestive of increased proteolysis. This increase in tau breakdown products was associated with TUNEL- and activated caspase-3-positive neurons identified histologically. These studies show that increases in soluble three repeat tau can be responsible for FTD in cases with tau gene mutations in the intronic region immediately adjacent to the stem loop in exon 10. These cases of FTD have tau isoforms (without exon 3 inserts) that do not form abnormal aggregates and appear more prone to proteolysis. The increase in tau proteolysis was associated with increased evidence of apoptosis. This mechanism of neurodegeneration may be more applicable to the majority of FTD cases, which do not accumulate insoluble tau deposits.

Brain and Development (3)


http://www.sciencedirect.com/science/article/B6T50-3SV3PJ8-4/2/48f3df10b9ae4b8914455b77a42d77f9
On dystrophin gene analysis by multiplex polymerase chain reaction (PCR), 76 of 130 (58.5%) Japanese patients with Duchenne muscular dystrophy had a deletion or duplication in genomic DNA. Of the remaining 54 patients who had no identifiable gene mutations, muscle biopsy tissue was available in 16 for RNA extraction. The full length of the coding regions of dystrophin cDNA was amplified in 10 fragments by reverse transcription nested PCR (RT-PCR). Five of 16 patients (31%) had dystrophin cDNA of abnormal size. One patient had a deletion, and two duplications that were not covered by multiplex PCR; one an exon-skipping of exon 51 caused by a 5' consensus splice site mutation of intron 51, and one 172 bp or 202 bp insertion in the cDNA between exon 25 and 26. Nested RT-PCR from the total RNA extracted from muscle biopsy was useful for screening patients who had no identifiable gene abnormality by multiplex PCR.

http://www.sciencedirect.com/science/article/B6T50-3W0NC53-C/2/3dbdda46e49bcc43fecd87765bc4a6cc

We report a rare case of intractable frontal lobe epilepsy with mental deterioration, in which the measles virus gene was detected from the cerebrospinal fluid (CSF) and peripheral mononuclear cells (PBMC) obtained 9 years after the first epileptic episode using reverse transcriptase-polymerase chain reaction (RT-PCR). The patient had been immunized with an attenuated measles vaccine and had no history of clinically apparent acute measles infection. However the analysis of the sequence of the PCR product from CSF showed the circulating wild strain genotype at the time when the patient complained of his first epileptic episode.

http://www.sciencedirect.com/science/article/B6T50-44KDHY-1T/2/e2e4a6663957372da9bd9ba8f76f

Rett syndrome (RTT) is an X-linked dominant neurological disorder, which appears to be the most common genetic cause of profound combined intellectual and physical disability in Caucasian females. This syndrome has been associated with mutations of the MECP2 gene, a transcriptional repressor of unknown target genes. We report a detailed mutational analysis of a large cohort of RTT patients from the UK and Italy. This study has permitted us to produce a hot spot map of the mutations identified. Bioinformatic analysis of the mutations, taking advantage of structural and evolutionary data, leads us to postulate the existence of a new functional domain in the MeCP2 protein, conserved among brain-specific regulatory factors.

Brain Research Bulletin (4)

There is growing evidence that Vitamin D3 (1,25-dihydroxyvitamin D3) is involved in brain development. We have recently shown that the brains of newborn rats from Vitamin D3 deficient dams were larger than controls, had increased cell proliferation, larger lateral ventricles, and reduced cortical thickness. Brains from these animals also had reduced expression of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor. The aim of the current study was to examine if there were any permanent outcomes into adulthood when the offspring of Vitamin D3 deficient dams were restored to a normal diet. The brains of adult rats were examined at 10 weeks of age after Vitamin D3 deficiency until birth or weaning. Compared to controls animals that were exposed to transient early Vitamin D3 deficiency had larger lateral ventricles, reduced NGF protein content, and reduced expression of a number genes involved in neuronal structure, i.e. neurofilament or MAP-2 or neurotransmission, i.e. GABA-A[alpha]4. We conclude that transient early life hypovitaminosis D3 not only disrupts brain development but leads to persistent changes in the adult brain. In light of the high incidence of hypovitaminosis D3 in women of child-bearing age, the public health implications of these findings warrant attention.


The expression of neuronal nitric oxide synthase (nNOS) and the cGMP-dependent protein kinases cGKI and cGKII in rat cerebellum was evaluated at different developmental stages by quantitative RT-PCR and Western blotting. mRNAs coding for these proteins were detected in the cerebella of rats aged 7, 14 and 21 days. Expression levels, nevertheless, varied significantly at each of these developmental stages. While nNOS and cGKI mRNA levels steadily increased during development, cGKII mRNA showed a different behaviour pattern, with similar levels observed on postnatal days 7 and 14 and increased levels noted on postnatal day 21. Moreover, protein expression profiles for nNOS and cGKI showed similar patterns to the mRNAs encoding these proteins. Our results reveal the developmental regulation of the expression of these proteins in the cerebellum, giving rise to higher levels as the cerebellum matures.


To understand graft rejection in cell based therapies for brain repair we have quantified IL-1[beta], IL-2, IL-4, IL-10, IL-12p40, IFN-[gamma] and TNF-[alpha] mRNA levels using real-time PCR, at days 4, 14, and 42 post-transplantation, in rats engrafted with syngeneic, allogeneic, concordant and discordant xenogeneic neural tissues. In addition, in the discordant xenografts immunohistochemistry and in situ hybridization were applied to detect local expression of IFN-[gamma], TNF-[alpha], IL-10 and TGF-[beta]. Allografts remained non-rejected but expressed IL-1[beta], TNF-[alpha] and IL-4 transcripts but not IL-12p40 and IFN-[gamma]. Xenografts
demonstrated distinct cytokine profiles that differed from syngeneic and allogeneic grafts. Non-rejected discordant xenografts contained higher levels of TNF-[alpha] transcripts and lower levels of IL-2 transcripts than the rejected ones at day 42. Discordant xenografts displayed a stronger and earlier expression of IL-1[beta] and TNF-[alpha], followed by T-helper 1 and T-helper 2 associated cytokine expression. The number of cells expressing mRNA encoding TNF-[alpha] and TGF-[beta] was significantly increased over time in the discordant group. In conclusion, the immunological disparity of the implanted tissue explains survival rates and is associated with different cytokine profiles. In allografts, a chronic inflammatory reaction was detected and in xenogeneic grafts a delayed hypersensitivity like reaction may be involved in rejection.


We reported previously that many neurodegenerative changes characteristic of apoptosis could be induced by a short fragment of [beta]-amyloid protein, A[beta]31-35, in cultured newborn mice cortical neurons, and that these changes were accompanied with alterations in expression of some genes. This study was designed to examine whether the apoptotic processes and related gene modulations in this model could be affected by coadministration of carbachol by electrophoretic analysis for DNA ladder formation and by RT-PCR assays for genomic modulation. The results showed that (1) simultaneous incubation with carbachol dose- and time-dependently blocked the specific DNA ladder formation induced by exposure to A[beta]31-35 and (2) the A[beta]31-35-induced downregulation of bcl-2 and upregulations of bax, p53, and c-fos genes were reversed or ameliorated by the coadministration of carbachol. It is proposed that A[beta]31-35-induced apoptosis can be prevented by carbachol through mechanisms that modulate the expression of related genes.

Brain Research Protocols  (15)


http://www.sciencedirect.com/science/article/B6T3N-49RCN34-1/2/cc66d489f5483758e0f03a1a7925b39

Detecting and quantifying generalized mitochondrial heteroplasmy is essential if the field of mitochondrial genetics is to advance in the arena of complex genetic disorders. The majority of techniques used to detect and quantify mitochondrial heteroplasmy focus on a known mutation or polymorphism. The necessity of knowing the mitochondrial DNA (mtDNA) change beforehand means that non-specific heteroplasmy in general cannot be assessed. In this study, we assessed the extent that denaturing high-performance liquid chromatography (dHPLC) could detect and quantify mitochondrial heteroplasmy from cerebrospinal fluid (CSF). Although we used a known polymorphism to assess reliability and sensitivity of this technique, a distinct advantage to using dHPLC for heteroplasmy detection is that the entire fragment is screened for variability and any
unique fragments will be detected regardless of the placement or type of change. Our results demonstrate that dHPLC can consistently and reliably detect mitochondrial heteroplasmy in a CSF sample down to 0.01%. In addition, the level of heteroplasmy was consistent with peak height for each homoduplex, giving a reliable method to quantify level of heteroplasmy.


Comparing female and male brain structures reveals a variety of sex differences in many vertebrates. Some of these differences are thought to be induced during the fetal period by the effect of steroid hormones produced in the gonads. Not much is known about molecular mechanisms involved in gender-specific development of the brain. We have taken a broad approach to isolate sex-specific genes from 18.5 days post coitum brain (A. Eriksson, C. Wahlestedt and K. Nordqvist. 1999. Isolation of sex-specific cDNAs from fetal mouse brain using mRNA differential display and representational difference analysis. Mol. Brain Res., 74, 91-97). Female and male mouse brains were screened with the signal peptide differential display, developed in our laboratory, and with a modified representational difference analysis of cDNA. The resulting sex-specific fragments were verified by semi-quantitative RT-PCR. Here we describe these methods in detail.


Rapid cloning of 5' and 3'-cDNA ends polymerase chain reaction (5'-/3'-RACE-PCR) is useful to determine unknown 5' and 3'-cDNA termini. Even if the method can yield complete cDNA sequences within a couple of days, the RACE procedure bears some characteristic traps and often results in amplification of unspecified PCR-products. Here we used improved 5'- and 3'-RACE-PCR protocols to obtain the complete cDNA sequence of the G-protein-coupled receptor kinase 6 (GRK6) from a rat brain cDNA library. The use of an anchored oligo-(dT)16-V-primer in the cDNA synthesis, the addition of single-sided PCR steps prior to the RACE-PCRs and the optimization of the dA-tailing reaction conditions in 5'-RACE enhanced RACE-PCR efficiency. Taken together, the method is a tool to determine unknown 5' and 3'-cDNA ends and enables the detection of different transcription initiation sites and mRNA splice variants even from small tissue samples like distinct brain regions. The extensive troubleshooting section discusses typical problems of each substep and contains additional references for support protocols. Themes: Cell biology, neurotransmitters, modulators, transporters, and receptors. Topics: Gene structure and function: general, second messengers and phosphorylation.

Gamma-amino butyric acid is the major inhibitory neurotransmitter in the brain. GABA transporters (GATs) remove GABA from the synaptic cleft. Till now, five distinct GABA transporters have been cloned and termed consecutively GAT1 to GAT4 and vGAT. To study the mechanisms by which tolerance and dependence associated with drugs enhancing GABAergic transmission is brought upon we analysed the mRNA expression levels of GATs in various brain regions under different conditions. In this paper, we describe our protocol for measurement of GAT3 mRNA expression, and its validation through control experiments for the various steps. We performed competitive reverse transcription and polymerase chain reaction (RT-PCR) with a competitor cRNA as internal standard. Different amounts of competitor cRNA were added to total RNA prepared from different tissue samples, reverse-transcribed and PCR amplified. The PCR amplification gave two products: the GAT wild type fragment and the competitor fragment. PCR products were separated by gel electrophoresis and band intensities were determined from which the relative and absolute abundance of GAT3 mRNA was calculated by regression analysis. Validation experiments in our laboratory showed a 6% intra-assay and a 15% inter-assay variability of this method. Themes: Neurotransmitters, modulators, transporters, and receptors

Topics: GABA, uptake and transporters


Reverse transcription-polymerase chain reaction (RT-PCR) is a powerful tool to detect specific gene expression from a small amount of tissue, which is superior to the traditional RNA assays such as Northern blotting and in situ hybridization (ISH) in terms of sensitivity. However, conventional RT-PCR is not suitable for quantification due to its exponential nature. Recently, a real-time quantitative PCR method has been developed to overcome the weak points of RT-PCR, e.g. quantification. Here we describe the use of real-time quantitative PCR using a fluorescent TaqMan probe, to study the regional differences in expression of glutamate receptor subunit/subtype genes (NR1, NR2A, GluR2, KA2, mGluR1, mGluR7) in the central vestibular system including the vestibular nucleus complex, inferior olive and cerebellar flocculus. We found that real-time quantitative PCR yielded similar results to other techniques such as ISH but offered several advantages in terms of relative speed and ability to detect low levels of gene expression. We suggest that real-time quantitative PCR is a useful method to study gene expression for other neurotransmitter receptors in the vestibular brainstem and cerebellum, and is also expected to be more accurate to assess the changes in gene expression following any treatment.


The N-methyl--aspartate (NMDA)-selective subtype of ionotropic glutamate receptor is of importance in neuronal differentiation and synapse consolidation, activity-dependent forms of synaptic plasticity, and excitatory amino acid-mediated neuronal toxicity [Neurosci. Res. Program
NMDA receptors exist in vivo as tetrameric or pentameric complexes comprising proteins from two families of homologous subunits, designated NR1 and NR2(A-D) [Biochem. Biophys. Res. Commun. 185 (1992) 826]. The gene coding for the human NR1 subunit (hNR1) is composed of 21 exons, three of which (4, 20 and 21) can be differentially spliced to generate a total of eight distinct subunit variants. We detail here a competitive RT-PCR (cRT-PCR) protocol to quantify endogenous levels of hNR1 splice variants in autopsied human brain. Quantitation of each hNR1 splice variant is performed using standard curve methodology in which a known amount of synthetic ribonucleic acid competitor (internal standard) is co-amplified against total RNA. This method can be used for the quantitation of hNR1 mRNA levels in response to acute or chronic disease states, in particular in the glutamatergic-associated neuronal loss observed in Alzheimer's disease [J. Neurochem. 78 (2001) 175]. Furthermore, alterations in hNR1 mRNA expression may be reflected at the translational level, resulting in functional changes in the NMDA receptor.


http://www.sciencedirect.com/science/article/B6T3N-48B59FN-8/2/06c35607188a9853f7be5cf62db3a73f

The NMDA-selective ionotropic receptor constitutes one of the three principal classes of glutamate receptors within the mammalian brain. It plays key roles in neuronal differentiation and synapse consolidation, activity-dependent forms of synaptic plasticity, and excitatory amino acid-mediated neuronal toxicity [Lab. Invest., 68 (1993) 372-387]. NMDA receptors exist as multimeric complexes comprising proteins from two families, NR1 and NR2(A-D) [J. Biol. Chem., 271 (1996) 15669-15674]. Studies on recombinant receptors have revealed that while homomeric NR2 receptors are non-functional, co-expression of an NR1 with an NR2 subunit modulates the efficacy of the resulting channel [Nature, 357 (1992) 70-74]. The RT-PCR assay we describe here was developed to allow quantitation of all hNR2 transcripts in a single-tube PCR assay. Each hNR2 isoform is quantified on the basis of standard curves in which a known amount of synthetic ribonucleic acid competitor is co-amplified against total RNA. The protocol has been applied to the quantitation of hNR2 mRNA levels in autopsy brain. Used in conjunction with a method for the quantitation of hNR1 transcripts [Brain Res. Protoc., in press], a complete analysis of NMDA receptor mRNA expression can be obtained.


http://www.sciencedirect.com/science/article/B6T3N-42D2CD1-4/2/d7326b1fc5464f5e5e2266ce0ce98924de

Our laboratory has developed a one-step quantitative reverse transcription polymerase chain reaction (RT-PCR) procedure in which the reverse transcriptase enzyme and Taq DNA polymerase are combined in the one tube and a single, non-interrupted, thermal cycling program is performed. In the past, RT-PCR has been carried out with two separate steps: (1) reverse transcription of RNA to generate a cDNA pool and (2) polymerase chain reaction amplification of the cDNA. The two-step method can affect the accuracy of the procedure as the total number of manipulations is greater, thereby allowing a greater chance for pipetting errors. Quantitation by our method is achieved in a single reaction by the use of a competitive internal standard that is identical in sequence to the target RNA except for a deletion of 107 base pairs and uses identical primers and cycling conditions. Using this method, we have been able to quantify the amount of message of a G protein (Gz[alpha]), in small amounts of tissue, such as dorsal root ganglia, from...
embryonic as well as postnatal mice.


http://www.sciencedirect.com/science/article/B6T3N-402K9HS-G/2/e897aacbaeb53dfc2454e4568af15430

Differential gene expression plays an important role in normal development and pathophysiological conditions. The accurate quantitation of mRNA expression is critical to assess the differential gene expression. While a number of techniques, such as Northern analysis, (semi-)quantitative reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization, are available to measure the levels of mRNA expression, certain limitations exist, including the insensitive and inaccurate quantitation of mRNA expressed at low abundance. In the present study, we describe the application of a recently developed TaqMan real-time quantitative RT-PCR for the detection of interleukin-1[beta] (IL-1[beta]) mRNA expression in rat cortical tissue after a short duration of ischemia (i.e., ischemic preconditioning). The principle of the TaqMan real-time detection is based on the fluorogenic 5' nuclease assay that allows simple and rapid quantitation of a target sequence during the extension phase of PCR amplification. Using a cloned plasmid DNA as a standard and normalizing RNA samples with a housekeeping gene for the TaqMan real-time PCR, we detected the significant induction in absolute copy numbers of IL-1[beta] mRNA in the ipsilateral cortex after preconditioning, suggesting a potential role of this inflammatory cytokine in ischemic brain tolerance. Themes: Disorders of the nervous systemTopics: Ischemia


http://www.sciencedirect.com/science/article/B6T3N-46WSXWG-2/2/2/92e5ff39739dced56fb1f003ccf280ba

We describe a protocol for analysis of gene expression in single, acutely dissociated adult rat retinal ganglion cells using RT-PCR. Retrograde tracing of retinal ganglion cells from the superior colliculi was conducted using Fluorogold. Retinas were dissected and ganglion cells isolated using retinal layer separation (sandwiching). Single, fluorescently labelled retinal ganglion cells were aspirated using a micropipette and used for PCR. Two PCR protocols are described where single cell cDNA was analysed for TrkB and GAPDH or TrkB, TrkC, Ret, Met, ErbB2 and Beta-actin by multiplex-PCR. All five tyrosine kinase receptors were amplified from single retinal ganglion cells. The method will prove useful for the molecular characterization of adult retinal ganglion cells.


http://www.sciencedirect.com/science/article/B6T3N-44HYF22-C/2/2d65e5a10bf9a2d98792ebec7baae0ac
Aldose reductase (AR) and sorbitol dehydrogenase (SDH) are the enzymes constituting the polyol pathway, an alternate route of glucose metabolism. A wealth of experimental data has indicated the involvement of the polyol pathway in the pathogenesis of diabetic complications. However, there has been surprisingly little research on the relative abundance of SDH to AR in the tissues affected in diabetes. We therefore developed a competitive RT-PCR system to simultaneously determine the mRNA levels of these two enzymes in small amounts of samples, and studied their expression in Schwann cells isolated from adult rat sciatic nerves. Although both AR and SDH mRNA were expressed in the Schwann cells, the levels of SDH cDNA were much lower than those of AR cDNA. The induction of AR mRNA expression in the Schwann cells under hyperosmotic conditions was similarly detected by Northern blot analysis and our competitive RT-PCR method. The RT-PCR system developed in this study may be a useful tool in ascertaining the relative contributions of AR and SDH to the metabolic derangements resulting from the acceleration of polyol pathway activity in the target organ of diabetic complications.


http://www.sciencedirect.com/science/article/B6T3N-3XG1TBJ-1/2/a6a0df67f98ecd193c267149074bb2e8

The reverse transcription linked polymerase chain reaction (RT-PCR) is a powerful technique for detecting mRNAs of low abundance while enabling distinction between homologous mRNAs such as family members and between alternative splice variants. We utilized this technique for quantitative analysis of expression of nine fibroblast growth factor (FGF) and four FGF receptor (FGFR) family genes in mouse brain during development and adulthood. The primer sets and reaction conditions for each family member were optimized for efficient amplification, and the amplified products were detected by hybridization with specific probes to ensure specificity. To achieve quantitative measurement, serial concentrations of the cloned cDNAs were simultaneously amplified and the results were used to titrate the amount of mRNA in the samples. Since FGF family has been recently recognized to be important in various functions of central nervous system and the protocol described here is directly applicable for a variety of small tissue samples, this protocol is very helpful in understanding the involvement of FGF family in various physiological phenomena.


The P2X receptor is a receptor-gated cationic channel that responds to ATP. The quantification of P2X mRNA expression in dorsal root ganglion (DRG) provides important information for neuropathic pain studies. We developed a rapid and sensitive external-standard-based real-time quantitative PCR assay for the quantification of mRNA of P2X receptors in mouse tissue samples. The assay uses a double-stranded DNA fluorescent dye, SYBR Green I, to continuously monitor product formation with a GeneAmp 5700 Sequence Detection System (PE Applied Biosystems). To establish the quantitative PCR amplification in a wide range of target transcripts, optimum parameters of primer sequences, concentrations of primers and/or templates, and PCR thermal protocols were experimentally determined. We also tested the reliability of this method in established experimental murine models, which were made by ligation or cutting down of the sciatic nerve. The parameters defined in this assay should be applicable to the quantification of other types of pain models and other tissue samples of mouse.
Defining molecular repertoires within virally infected tissues of the nervous system may provide insight into the pathogenesis of, and immunity to, neurotropic viruses. Here we report the application of such a method, namely mRNA differential display (DD), to the identification of mRNAs that are expressed at different levels in herpes simplex virus (HSV) infected nervous tissue from immunocompetent and CD8+ lymphocyte depleted mice. Small amounts of input RNA can be used by DD, making the method ideal for experiments based on murine sensory ganglia (DRG), which on average yield less than 0.5 [mu]g of total RNA. In the current work, DD facilitated the identification of a mRNA whose abundance in HSV-infected ganglia, based on Northern blot analysis, was reduced in mice depleted of CD8+ cells. The cloned product of this mRNA was of particular interest to our research as sequence data strongly suggested that it represented the murine homologue of the [alpha] chain of a G protein termed Golf. This G protein had not previously been reported from dorsal root ganglial tissue. RT-PCR confirmed the presence of Golf in DRG and in situ hybridization studies localised this molecule to primary sensory neurons. These data indicate that DD is sufficiently robust to be applied to the study of virus pathogenesis within the nervous system.


Reverse-transcribed polymerase chain reaction (RT-PCR) can quantify gene transcripts at low levels and in small samples. Semi-quantitative and quantitative RT-PCR has significant advantages over traditional RNA assays, such as Northern blotting and ribonuclease protection assay. However, owing to the exponential nature of PCR, considerable effort is required to verify linearity of the reaction. Thus, care must be taken to detect small but physiologically relevant changes in gene expression. Using a rapid and highly sensitive RT-PCR method, TaqMan real-time RT-PCR, we determined agonist-induced changes in rat mu opioid receptor (MOR) mRNA levels in cultured cells and compared our results with those obtained by radiolabeled quantitative RT-PCR, which is also highly sensitive but much more time-consuming than TaqMan RT-PCR. Both methods showed up-regulation of agonist-induced MOR. TaqMan RT-PCR showed a similar sensitivity to radiolabeled quantitative RT-PCR and is suitable for the measurement of large numbers of samples. Moreover, no need for radiolabeled compounds is also an advantage of TaqMan PCR. This protocol will probably be useful for quantifying MOR in animal and human tissues.
Astrocytes are characterized by extensive gap junctional intercellular communication (GJIC) mediated primarily by channels composed of connexin43. In contrast, C6 glioma cells are deficient in connexin expression and gap junctional communication. Transfection of these glioma cells with connexin cDNAs results in changes in cellular phenotype following increased GJIC. Specifically, connexin expression correlates with reduced cellular proliferation and tumorigenicity. To characterize the role of gap junctions in this growth control, we have screened for changes in gene expression by differential display. We have observed that these changes in GJIC are associated with changes in expression of several genes, including those coding for a number of secreted factors which may play a role in modulating the tumor phenotype of these cells. These include the immediate early gene cyp61, osteopontin and the KC gene (murine homologue of the human gro gene).


Several reports show that behavioural and physiological components of the acute phase reaction can be conditioned. However, the mechanisms responsible for these effects remain obscure. The underlying assumption that the changes observed in conditioned animals are dependent on a conditioned production of cytokines has never been demonstrated. In the present study, the possibility of conditioning the production of cytokines or molecules implicated in their signalling pathways was tested by submitting mice to conditioned taste aversion with a new saccharin taste paired with intraperitoneal (i.p.) injections of lipopolysaccharide (LPS, 0.83 [mu]g/g) or peptidoglycan (PGN, 20 [mu]g/g). After two conditioning sessions, conditioned mice developed a clear aversion to saccharine that was not associated with activation of genes of the cytokine network either at the periphery, or in the hypothalamus, as demonstrated by a macroarray approach and confirmed by real time RT-PCR. In contrast, there was an activation of the genes coding for nuclear factor kappa B (NF[kappa]B) and mitogen activated protein kinase (MAPK) signalling pathways in the spleen and to a lesser extent in the hypothalamus. This modulation of the NF[kappa]B and MAPK signalling pathways is interpreted in terms of a possible conditioned
The beta-2-adrenergic receptor (β2AR) is expressed by most lymphocyte populations and binds the sympathetic neurotransmitter norepinephrine (NE). Stimulation of the β2AR is reported to be the primary mechanism by which signals from the sympathetic nervous system influence both cell-mediated and humoral immunity. We report here that body/organ weights, lymphoid organ cell number/phenotype/histology, the contact sensitivity response, and the amount, avidity, and isotype of antibody resulting from a T cell-dependent antibody response in β2AR deficient mice (β2AR-/- mice) were all similar to measures made in β2AR+/+ mice. Other members of the adrenergic receptor family did not appear to compensate for the absence in β2AR expression. In contrast, β2AR-/- B cells cultured in vitro were unable to respond to NE in a manner similar to β2AR+/+ B cells. Thus, mice in which expression of the β2AR gene is defective from early development to adulthood may no longer require that NE stimulate the β2AR to maintain immune homeostasis, and this may be due to a non-adrenergic mechanism that provides compensation in vivo.


Human T cell leukemias can arise from oncogenes activated by specific chromosomal translocations involving the T cell receptor genes. Here we show that five different T cell oncogenes (HOX11, TAL1, LYL1, LMO1, and LMO2) are often aberrantly expressed in the absence of chromosomal abnormalities. Using oligonucleotide microarrays, we identified several gene expression signatures that were indicative of leukemic arrest at specific stages of normal thymocyte development: LYL1+ signature (pro-T), HOX11+ (early cortical thymocyte), and TAL1+ (late cortical thymocyte). Hierarchical clustering analysis of gene expression signatures grouped samples according to their shared oncogenic pathways and identified HOX11L2 activation as a novel event in T cell leukemogenesis. These findings have clinical importance, since HOX11 activation is significantly associated with a favorable prognosis, while expression of TAL1, LYL1, or, surprisingly, HOX11L2 confers a much worse response to treatment. Our results illustrate the power of gene expression profiles to elucidate transformation pathways relevant to human leukemia.


R-etodolac, a nonsteroidal anti-inflammatory drug, inhibits the progression of CWRSA6 androgen-independent and LuCaP-35 androgen-dependent prostate cancer xenograft growth through downregulation of cyclin D1 expression via the PPAR[gamma] pathway. PPAR[gamma] protein degradation, observed post-R-etodolac treatment, resulted from phospho-MAP kinase (p44/42) induction by R-etodolac negatively regulating PPAR[gamma] function. Negative regulation of PPAR[gamma] was overcome by a combination regimen of R-etodolac with the HER-kinase axis inhibitor, rhuMab 2C4, which demonstrated an additive antitumor effect. We further show that the inhibition of HER-kinase activity by rhuMab 2C4 is sufficient to inhibit PPAR[gamma] protein degradation. This study introduces a novel concept of an in vivo crosstalk between the HER-kinase axis and PPAR[gamma] pathways, ultimately leading to negative regulation of PPAR[gamma] activity and tumor growth inhibition.


The mechanisms of drug resistance in cancer are poorly understood. Serial analysis of gene expression (SAGE) profiling of cisplatin-resistant and sensitive cells revealed many differentially expressed genes. Remarkably, many ECM genes were elevated in cisplatin-resistant cells. COL6A3 was one of the most highly upregulated genes, and cultivation of cisplatin-sensitive cells in the presence of collagen VI protein promoted resistance in vitro. Staining of ovarian tumors with collagen VI antibodies confirmed collagen VI expression in vivo and suggested reorganization of the extracellular matrix in the vicinity of the tumor. Furthermore, the presence of collagen VI correlated with tumor grade, an ovarian cancer prognostic factor. These results suggest that tumor cells may directly remodel their microenvironment to increase their survival in the presence of chemotherapeutic drugs.


A CpG island DNA methylator phenotype has been postulated to explain silencing of the hMLH1 DNA mismatch repair gene in cancer of the microsatellite mutator phenotype. To evaluate this model, we analyzed methylation in CpG islands from six mutator and suppressor genes, and thirty random genomic sites, in a panel of colorectal cancers. Tumor-specific somatic hypermethylation was a widespread age-dependent process that followed a normal Gaussian distribution. Because there was no discontinuity in methylation rate, our results challenge the methylator phenotype hypothesis and its hypothetical pathological underlying defect. We also show that the mutator phenotype dominates over the gradual accumulation of DNA hypermethylation in determining the genotypic features that govern the phenotypic peculiarities of colon cancer of the mutator pathway.
Cancer Detection and Prevention  


http://www.sciencedirect.com/science/article/B6X28-45MCS85-B/2/3e8c25ad6a8b719f7042eaed7d1e7f30

The detection of circulating cancer cells in the bone marrow (BM) and peripheral blood (PB) of patients with solid tumors may be useful for disease staging. To this aim, we evaluated the expression of the mammaglobin gene by reverse transcriptase polymerase chain reaction (RT-PCR) in 60 patients with breast cancer. Moreover, several controls were examined to test the specificity of this marker. The positive cases included 23.6% of the patients with and 9% of those without metastasis. Only 4/60 negative controls analyzed were positive by PCR. Our results show high specificity and a good correlation with disease status.


http://www.sciencedirect.com/science/article/B6X28-4772XM6-2/2/8333263edf0a0949d6c7384bbebdd6ca

To investigate the etiological association of allelic loss at chromosomal regions containing tumor suppressor genes (TSGs) in non-small cell lung cancer (NSCLC) in Taiwan, we examined 48 microdissected NSCLC samples for loss of heterozygosity (LOH) at nine loci where TSGs are localized nearby. The associations of LOH at each locus with clinicoparameters and prognosis were also examined. The frequent LOH was observed using markers, D3S1285 near the FHIT gene (58.3%), D17S938 near the p53 gene (56.7%), D9S925 near the p16 gene (54.5%), and D13S153 near the RB gene (47.6%). The occurrence of LOH at each TSG locus was compared with the patients' clinicoparameters. The incidence of LOH at D17S938 (p53 gene) and D3S4545 (VHL gene) was significantly higher in squamous carcinoma tumors than in adenocarcinoma tumors (P=0.003 and 0.024, respectively). LOH of these two loci also occurred frequently in tumors from smoker patients compared to that from nonsmoker patients (P=0.013 and 0.025, respectively). LOH at D13S153 (RB gene) was also associated with smoking (P=0.008). In addition, the prognostic analyses indicated that the patients with LOH at D18S535 (18q21, near the SMAD2/4 gene) had significantly longer post-operative survival time compared to those without LOH (P=0.03). Our results suggested that LOH at FHIT, p53, and p16 genes may occur frequently in NSCLC patients in Taiwan. In addition, LOH at p53, RB, and VHL may associate with smoking or squamous carcinoma patients and LOH at SMAD2/4 may be correlated with better prognosis.


http://www.sciencedirect.com/science/article/B6X28-4D67948-
Synovial sarcoma (SS) is characterized by the t(X;18)(p11.2;q11.2) chromosomal translocation, which results in generating either SYT-SSX1, SYT-SSX2 or, infrequently, SYT-SSX4 fusion gene. The ratio of SYT-SSX1:SYT-SSX2 fusions is close to 2:1 in the majority of studies, and SYT-SSX2 fusion has been only rarely observed in biphasic SS. In the present study, we compared two series of patients with SS, Slovenian (37 cases) and Dutch (14 cases), with respect to clinical, pathological and molecular findings. The two groups did not differ with regard to clinicopathological features. Whereas the frequency of different SYT-SSX fusions in the Dutch group was similar to that reported in the literature, we found an unexpectedly high number of tumors with SYT-SSX2 fusion in the Slovenian group. The ratio of SYT-SSX1:SYT-SSX2 fusion was 7:18 for monophasic and 2:7 for biphasic tumors in the Slovenian group. This distribution differs significantly from that observed in the Dutch group in the present study (P = 0.041) as well as from data reported in the recent large multi-institutional study on 243 patients (P = 0.0001). Our findings indicate possible geographical differences in the frequency of two SYT-SSX fusion transcripts in patients with synovial sarcoma.


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http://cebp.aacrjournals.org/cgi/content/abstract/13/12/2141
Objective: The progestagenic milieu of pregnancy and oral contraceptive use is protective against epithelial ovarian cancer. A functional single nucleotide polymorphism in the promoter of the progesterone receptor (+331A) alters the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. In this study, we sought to determine whether this polymorphism affects ovarian cancer risk. Methods: The +331G/A polymorphism was genotyped in a population-based, case-control study from North Carolina that included 942 Caucasian subjects (438 cases, 504 controls) and in a confirmatory group from Australia (535 cases, 298 controls). Logistic regression analysis was used to calculate age-adjusted odds ratios (OR). Results: There was a suggestion of a protective effect of the +331A allele (AA or GA) against ovarian cancer in the North Carolina study [OR, 0.72; 95% confidence interval (95% CI), 0.47-1.10]. Examination of genotype frequencies by histologic type revealed that this was due to a decreased risk of endometrioid and clear cell cancers (OR, 0.30; 95% CI, 0.09-0.97). Similarly, in the Australian study, there was a nonsignificant decrease in the risk of ovarian cancer among those with the +331A allele (OR, 0.83; 95% CI, 0.51-1.35) that was strongest in the endometrioid/clear cell group (OR, 0.60; 95% CI, 0.24-1.44). In the combined U.S.-Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline), the +331A allele was significantly associated with protection against this subset of ovarian cancers (OR, 0.46; 95% CI, 0.23-0.92). Preliminary evidence of a protective effect of the +331A allele against endometriosis was also noted in control subjects (OR, 0.19; 95% CI, 0.03-1.38). Conclusions: These findings suggest that the +331G/A progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid/clear cell ovarian cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/11/11/1394

Hybrid Capture 2 Test using probe B (HC2-B) is a clinical test for the detection of 13 human papillomavirus (HPV) types associated with cervical cancer (oncogenic types), but the potential clinical significance of HC2-B cross-reactivity with untargeted (nononcogenic) HPV types has not been fully evaluated. Thus, HC2-B test results on 954 clinical cervical specimens from a population-based natural history study of HPV in Costa Rica were compared with the data from testing of the same specimens twice by HPV type-specific MY09/MY11 L1 consensus primer PCR. Specimens positive by PCR for single HPV types not targeted by HC2-B were used for determining type-specific cross-reactivity. Effects of cross-reactivity on clinical performance were estimated by calculating sensitivity and specificity with and without cross-reactivity for the detection of high-grade cervical lesions. HC2-B tested positive for single infections by untargeted (cross-reactive) types 11, 53, 61, 66, 67, 70, 71, and 81. Cross-reactivity was strongly associated with PCR signal strength (PTrend = 0.0001) and cervical abnormalities (P = 0.0002, Pearson χ²). We estimated that HC2-B cross-reactivity resulted in minor changes in screening performance. Clinical sensitivity increased from 84.3% to 87.9%, clinical specificity decreased from 89.6% to 88.1%, and referral rates increased from 11.7% to 13.2% for detection of [≥]cervical intraepithelial neoplasia grade 2. The clinical effect of cross-reactivity varied by cytologic interpretation. Among women with normal cytologic interpretations, cross-reactivity significantly improved the accuracy of identifying cytologically nonevident histology of [≥]cervical intraepithelial neoplasia grade 2 because of increased sensitivity with maintained specificity. However, among women with equivocal or mildly abnormal cytologic interpretations, cross-reactivity decreased the accuracy of HPV testing because of substantial decreases in specificity. In summary, cross-reactivity with nononcogenic HPV types had little effect on the overall clinical performance of HC2-B as a general screening test, but reduction of cross-reactivity might improve the performance of HPV testing for triage of equivocal or mildly abnormal cytologic
In large active cohort studies of women investigating human papillomavirus (HPV) and cervical neoplasia, many women will be HPV-negative at all time points and testing of all their cervical specimens is an inefficient use of laboratory resources. The aim of this pilot study was to evaluate whether pooling cervical specimens from the same woman might provide a useful pretest of specimens from women unlikely to have high-grade cervical neoplasia or significant HPV exposure. We selected women (n = 187) participating in the Guanacaste Project for whom we already had HPV testing data on all their specimens from multiple visits (median = 8 visits), who were HPV DNA-negative at enrollment and at their 5- to 7-year exit from the cohort, and had no evidence of high-grade cervical neoplasia. Equal aliquots of cervical specimens from these women were pooled to create a proportional pooled specimen. Aliquots of pooled specimens were tested in a masked fashion by MY09/11 L1 consensus primer PCR. Second aliquots of some pooled specimens (n = 83) were included to assess the reliability of pooled testing. Results were compared with the predicted (expected) results based on the obtained test results of the individual specimens collected at interim visits. There was good overall agreement between observed and expected HPV DNA positivity, with a $\kappa$ of 0.63 [95% confidence interval (95% CI), 0.51-0.75] and a percent agreement of 83.4% (95% CI, 77.3-88.5%) although the HPV DNA positivity in the pooled specimen was less than expected (P = 0.001). The agreement between observed and expected HPV DNA positivity was related to the number of aliquots pooled, suggesting that positivity was related to viral genome concentrations. The $\kappa$ and percent agreement for intra-batch reliability of testing pooled specimens were 0.68 (95% CI, 0.53-0.84) and 84.3% (95% CI, 74.7-91.4%), respectively. We conclude that pooling specimens and testing by PCR may be useful for discriminating HPV DNA-positive from completely negative specimen sets in women who are likely to have been HPV DNA-negative.


http://cebp.aacrjournals.org/cgi/content/abstract/11/1/131

The ideal technology for screening single-nucleotide polymorphisms requires high throughput with minimal cost per sample, minimal usage of valuable DNA resources, and maximal flexibility for assessment of new polymorphisms. We demonstrate here the feasibility of kinetic allele-specific PCR with DNA pooling (S. Germer et al., Genome Res., 10: 258-266, 2000) in a population study that satisfies all of the mentioned criteria and offers a powerful new tool for detecting meaningful polymorphic differences in candidate gene association studies and genome-wide linkage disequilibrium scans. Three individuals prepared pooled DNA samples from 269 individuals separated into three racial/ethnic groups: Caucasians (n = 56), African-Americans (n = 86), and Hispanics (n = 127). We used kinetic allele-specific PCR to determine the allele frequencies of the common paraoxonase 1 polymorphism, PON1 Q191R, in these pools. Paraoxonase 1 is a critical enzyme for inactivating neurotoxic intermediates in the metabolism of organophosphates. In a blinded test of the technology, these nine pooled DNA samples were sent to Roche for genotyping by kinetic allele-specific PCR. The allele frequencies found were 0.266 {+/-} 0.011, 0.386 {+/-} 0.011, and 0.617 {+/-} 0.010, respectively, which were comparable.
to the frequencies of 0.269, 0.403, and 0.622 determined by PCR-restriction fragment length polymorphism analysis. These same samples were genotyped on two kinetic PCR platforms from different manufacturers, using three different DNA polymerases. The results were comparable between both platforms and among all three polymerases. The results demonstrate a powerful new technology for determining frequencies of single-nucleotide polymorphisms in an epidemiological study.


http://cebp.aacrjournals.org/cgi/content/abstract/13/2/285

5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, diverting metabolites toward methylation reactions or nucleotide synthesis. Using data from an incident case-control study (1608 cases and 1972 controls) we investigated two polymorphisms in the MTHFR gene, C677T and A1298C, and their associations with risk of colon cancer. All of the combined genotypes were evaluated separately, and the 1298AA/677CC (wild-type/wild-type) group was considered the reference group. Among both men and women, the 677TT/1298AA (variant/wild-type) genotype was associated with a small reduction in risk [men: odds ratio (OR), 0.7, 95% confidence interval (CI), 0.5-1.0; women: OR, 0.8, 95% CI, 0.5-1.2]. However, the 677CC/1298CC (wild-type/variant) genotype was associated with a statistically significant lower risk among women (OR, 0.6; 95% CI, 0.4-0.9) but not men. When the polymorphisms were considered individually, for A1298C a significant risk reduction associated with the homozygous variant CC genotype was seen among women only (OR, 0.6; 95% CI, 0.5-0.9), and nonstatistically significant reduced risks were observed for the variant 677 TT genotypes among both men and women. Stratification by nutrient intakes showed inverse associations with higher intakes of folate, vitamin B2, B6, B12, and methionine among women with the MTHFR 677CC/1298AA genotypes, but not those with 677TT/1298AA. We observed opposite risk trends for both MTHFR variants, depending on whether women used hormone-replacement therapy or not (P for interaction = <.01). In summary, this study supports recent findings that the MTHFR A1298C polymorphism may be a predictor of colon cancer risk and have functional relevance. The possible interaction with hormone-replacement therapy warrants additional investigation.


http://cebp.aacrjournals.org/cgi/content/abstract/12/11/1130

Microsatellite instability (MSI) occurs in 10-20% of the sporadic colon carcinomas and appears to be primarily due to alterations in hMLH1 and hMSH2. Little is known about the role of diet in MSI-related colon carcinogenesis. We used data from a Dutch population-based case-control study on sporadic colon carcinomas (184 cases and 259 controls) to evaluate associations between dietary factors previously reported as being associated with colon cancer risk and MSI, hMLH1 expression, and hMLH1 hypermethylation. Red meat intake was significantly differently related to microsatellite instability-high (MSI-H) tumors compared with microsatellite instability-low/microsatellite stable (MSI-L/MSS) [odds ratio (OR), 0.3; 95% confidence interval (CI), 0.1-0.9]. It was inversely associated with MSI-H tumors when compared with the population-based controls (OR, 0.5; 95% CI, 0.2-1.2) and positively associated with MSI-L/MSS tumors (OR, 1.5; 95% CI, 0.9-2.6). A positive association was observed for alcohol intake with MSI-H tumors (OR, 1.9; 95% CI, 0.8-4.7). Fruit consumption seemed to especially decrease the risk of MSI-H tumors with hypermethylated hMLH1 (Methyl+ tumors) [Methyl+ versus controls: OR = 0.4 and 95% CI = 0.2-0.9; MSI-H tumors without hypermethylated hMLH1 (Methyl- tumors) versus controls, OR =
1.2 and 95% CI = 0.8-1.7; Methyl+ versus Methyl- tumors, OR = 0.2 and 95% CI = 0.1-0.9]. Most other evaluated dietary factors were not distinctively associated with a specific MSI or hMLH1 methylation status. Our data suggest that red meat consumption may enhance the development of MSI-L/MSS carcinomas in particular, whereas alcohol intake appears to increase the risk of MSI-H tumors. Fruit consumption may especially decrease the risk of MSI-H carcinomas exhibiting epigenetically silenced hMLH1.


http://cebp.aacrjournals.org/cgi/content/abstract/12/9/815

Increased understanding of human papillomavirus (HPV) infection as the central cause of cervical cancer has permitted the development of improved screening techniques. To evaluate their usefulness, we evaluated the performance of multiple screening methods concurrently in a large population-based cohort of >8500 nonvirginal women without hysterectomies, whom we followed prospectively in a high-risk region of Latin America. Using Youden's index as a measure of the trade-off between sensitivity and specificity, we estimated the performances of a visual screening method (cervicography), conventional cytology, liquid-based cytology (ThinPrep), and DNA testing for 13 oncogenic HPV types. The reference standard of disease was neoplasia [IMG]=" BORDER="0"> cervical intraepithelial neoplasia grade 3 (CIN 3), defined as histologically confirmed CIN 3 detected within 2 years of enrollment (n = 90) or invasive cancer detected within 7 years (n = 20). We analyzed each technique alone and in paired combinations (n = 112 possible strategies), and evaluated the significance of differences between strategies using a paired Z test that equally weighted sensitivity and specificity. As a single test, either liquid-based cytology or HPV DNA testing was significantly more accurate than conventional cytology or cervicography. Paired tests incorporating either liquid-based cytology or HPV DNA testing were not substantially more accurate than either of those two test strategies alone. However, a possibly useful synergy was observed between the conventional smear and cervicography. Consideration of age or behavioral risk profiles did not alter any of these conclusions. Overall, we conclude that highly accurate screening for cervical cancer and CIN 3 is now technically feasible. The remaining vital issue is to extend improved cervical cancer prevention programs to resource-poor regions.


http://cebp.aacrjournals.org/cgi/content/abstract/12/10/970

An increased occurrence of colorectal cancer and its adenoma precursor is observed among individuals with low intakes or circulating levels of folate, especially if alcohol intake is high, although results have not been statistically significant in all studies. We examined folate and alcohol intake and genetic polymorphisms in methylenetetrahydrofolate reductase [MTHFR 667[-&gt;T (ala[-&gt;val) and MTHFR 1298A[-&gt;C (gln[-&gt;ala]) (associated with reduced MTHFR activity) and in alcohol dehydrogenase 3 [ADH3 (2-2) associated with decreased alcohol catabolism] in relation to risk of colorectal adenoma in the Health Professionals Follow-Up Study. Among 379 cases and 726 controls, MTHFR genotypes were not appreciably related to risk of adenoma, but a suggestive interaction (P = 0.09) was observed between MTHFR 677C[-&gt;T and alcohol intake; men with TT homozygotes who consumed 30+ g/day of alcohol had an odds ratio (OR) of 3.52 [95% confidence interval (CI), 1.41-8.78] relative to drinkers of [=]5 g/day with the CC/CT genotypes. ADH3 genotype alone was not appreciably related to risk, but its influence
was modified by alcohol intake. Compared with fast alcohol catabolizers [ADH3(1-1)] with low intakes of alcohol (≤5 g/day), high consumers of alcohol (30+ g/day) had a marked increase in risk if they had the genotype associated with slow catabolism [ADH3(2-2); OR, 2.94; 95% CI, 1.24-6.92] or intermediate catabolism [ADH3(1-2)] of alcohol (OR, 1.83; 95% CI, 1.03-3.26) but not if they were fast catabolizers [ADH3(1-1); OR = 1.27; 95% CI = 0.63-2.53). In addition, an increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1-137) was observed for those with the ADH3(2-2) genotype and high alcohol-low folate intake compared with those with low alcohol-high folate intake and the ADH3(1-1) genotype (P for interaction = 0.006). Our results indicate that high intake of alcohol is associated with an increased risk of colorectal adenoma, particularly among MTHFR 677TT and ADH3(2-2) homozygotes. The findings that alcohol interacts with a folate-related gene (MTHFR) and that the interaction between alcohol and ADH3 is stronger among those with low folate intake support the hypothesis that the carcinogenic influence of alcohol in the large bowel is mediated through folate status.


http://cebp.aacrjournals.org/cgi/content/abstract/12/6/477

Studies investigating human papillomavirus (HPV) viral load as a risk factor in the development of squamous intraepithelial lesions (SILs) and cancer have often yielded conflicting results. These studies used a variety of HPV viral quantitation assays [including the commercially available hybrid capture 2 (HC 2) assay], which differ in their ability to account for differences in cervical cell collection, linear dynamic range of viral load quantitation, and determination of type-specific versus cumulative viral load measures. HPV-16 and HPV-18 viral quantitation using real-time PCR assays were performed to determine whether type-specific viral load measurements that adjust for specimen cellularity result in a different association between viral load and prevalent SIL and cancer, compared with HC 2 quantitation (which does not adjust for cellularity or multiple infections). In general, HPV-16 viral load as measured by real-time PCR increased linearly with increasing grade of SIL while HPV-18 measured using similar techniques increased through low-grade SIL (LSIL), with HPV-18 viral load among high-grade SIL and cancers near the level of cytologically normal women. HC 2 viral load, using the clinical 1.0 pg/ml cut point, differentiated cytologically normal women from women with any level of cytological abnormality (normal versus >=LSIL) but did not change as lesion severity increased. There was no evidence for plateau of HC 2 at high copy numbers, nor was significant variability in total specimen cellularity observed. However, cumulative viral load measurements by HC 2, in the presence of multiple coinfections, overestimated type-specific viral load. Multiple infections were more common among women with no (32%) or LSIL (51%) [versus 23% in high-grade SIL/cancer], partially explaining the lack of a dose response using a cumulative HC2 viral load measure. The nonrandom distribution of multiple infections by case-control status and the apparent differential effect of viral load by genotype warrant caution when using HC 2 measurements to infer viral load associations with SIL and cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/13/8/1407

The production of estrogen from androgen via the estrogen biosynthesis pathway is catalyzed by aromatase P450 (CYP19). To assess the association between breast cancer risk and a
polymorphism at codon 39 Trp/Arg of the encoding gene, a case-control study was conducted at Aichi Cancer Center Hospital in Japan. Subjects were 248 histologically confirmed breast cancer patients and 603 hospital controls without cancer. Odds ratios (OR) and 95% confidence intervals (95% CI) were determined by logistic regression analysis. The allele frequency among controls was 3.8% for the C allele, and the OR (95% CI) of the polymorphism relative to TT genotype was 1.21 (0.69-2.14) for TC/CC genotypes combined. There was no association between CYP19 gene polymorphism and breast cancer risk in the study group as a whole, but homozygous and heterozygous carriers of the variant Arg allele showed a significantly increased risk of breast cancer among premenopausal women with a late age at first full-term pregnancy (OR 7.31, 95% CI 1.88-28.5) or a high body mass index (OR 2.77, 95% CI 1.12-6.87). Additional larger studies should be done to confirm that the rare CYP19 variant increases the risk of breast cancer among premenopausal Japanese women.


http://cebp.aacrjournals.org/cgi/content/abstract/12/9/838

Extensive mammographic density is heritable, strongly associated with increased breast cancer risk, and is influenced by sex hormone exposure. In a cross-sectional study of 181 pre- and 171 postmenopausal women without breast cancer, we examined the relationship of a functional polymorphism in catechol-O-methyltransferase (COMT; VAL[-gt]MET) to mammographic density and other risk factors for breast cancer. We hypothesized that individuals who inherited the low-activity form of COMT (COMT*2 allele) would have higher levels of breast density, presumably because of reduced inactivation/detoxification of catecholestrogens. Subjects were recruited across five categories of breast density. Risk factor information, anthropometric measures, and blood samples were obtained; sex hormone and growth factor levels were measured, and COMT genotypes determined. Mammograms were digitized and measured using a computer-assisted method. After adjustment for age and ethnicity, among pre- but not postmenopausal subjects, each low-activity COMT*2 allele was associated with lower levels of percentage breast density. The statistical significance of this association was lost after further adjustment for serum growth factors [growth hormone, insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-3 (IGFBP-3)], hormones [follicle-stimulating hormone (FSH) and progesterone], and body size (body mass index and waist:hip ratio). The low-activity COMT*2 allele was also associated, after adjustment for age and ethnicity in premenopausal women, with lower serum levels of IGF-1, higher levels of FSH and progesterone, and with a larger waist:hip ratio, body mass index, and subscapular skinfold. After adjustment for body size, the associations of genotype with IGFBP-3 and FSH were no longer significant. These findings indicate that COMT genotype is associated with several risk factors for breast cancer and suggest that the low-activity COMT*2 allele is associated with a reduced risk of breast cancer among premenopausal women.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/913

Somatic mutations of BRAF have been identified in both melanoma tumors and benign nevi. Germ line mutations in BRAF have not been identified as causal in families predisposed to melanoma. However, a recent study suggested that a BRAF haplotype was associated with risk of sporadic melanoma in men. Polymorphisms or other variants in the BRAF gene may therefore
act as candidate low-penetrance genes for nevus/melanoma susceptibility. We hypothesized that promoter variants would be the most likely candidates for determinants of risk. Using denaturing high-pressure liquid chromatography and sequencing, we screened peripheral blood DNA from 184 familial melanoma cases for BRAF promoter variants. We identified a promoter insertion/deletion in linkage disequilibrium with the previously described BRAF polymorphism in intron 11 (rs1639679) reported to be associated with melanoma susceptibility in males. We therefore investigated the contribution of this BRAF polymorphism to melanoma susceptibility in 581 consecutively recruited incident cases, 258 incident cases in a study of late relapse, 673 female general practitioner controls, and the 184 familial cases. We found no statistically significant difference in either genotype or allele frequencies between cases and controls overall or between male and female cases for the BRAF polymorphism in the two incident case series. Our results therefore suggest that the BRAF polymorphism is not significantly associated with melanoma and the promoter insertion/deletion linked with the polymorphism is not a causal variant. In addition, we found that there was no association between the BRAF genotype and mean total number of banal or atypical nevi in either the cases or controls.


http://cebp.aacrjournals.org/cgi/content/abstract/11/12/1611

We evaluated polymorphisms in methylenetetrahydrofolate reductase (MTHFR), folate intake and alcohol consumption in relation to risk of colon cancer in a population-based case-control study in North Carolina. The study included 555 cases (244 African Americans and 311 whites) and 875 controls (331 African Americans and 544 whites). Total folate intake of <400 versus [&gt;=]400 {micro}g/ day showed a weak positive association with colon cancer among both African Americans [adjusted odds ratio (OR) = 1.4, 95% confidence interval (CI) = 1.0-2.0] and whites (OR = 1.6, 95% CI = 1.2-2.2). No association was observed with use of alcohol. Compared with wild-type genotypes, there was no association between the low activity MTHFR codon 677 TT genotype and colon cancer, but the low activity codon 1298 CC genotype was inversely associated with colon cancer in whites (OR = 0.5, 95% CI = 0.3-0.9). Unlike previous studies, we did not observe a strong protective effect of the codon 677 TT low-activity genotype when folate intake was high. Instead, we observed an increased risk of colon cancer when folate intake was low for participants with wild- type genotypes. Adjusted ORs for the combined effects of codon 677 CC and codon 1298 AA genotypes and folate intake &lt;400 {micro}g/day were 1.9 (95% CI = 1.1-3.4) in African Americans and 2.5 (95% CI = 1.2-5.2) in whites. Our results suggest that variation at MTHFR codon 1298 (within the COOH-terminal region) may be more important for colon cancer than variation at codon 677 (NH2-terminal region), and in populations where folate intake is low, wild-type MTHFR activity may increase risk for colon cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/11/1/127

p53 is a transcription factor for Waf-1/p21, a cyclin-dependent kinase inhibitor. Certain polymorphic variants of Waf-1 and p53 have been evaluated for their association with cancer risk. Previous studies indicated that certain p53 polymorphisms confer an increased risk of breast cancer [odds ratios (ORs) and 95% confidence intervals (CIs) = 2.9, 1.4-6.3 Carcinogenesis (Lond.), 17: 1313, 1996; 2.5, 1.3-4.8 Cancer Epidemiol. Biomark. Prev., 6: 105, 1997; and 1.5, 1.1-2.0, Anticancer Res., 18: 2095, 1998). The primary objectives of this study were to test the
hypotheses that the serine variant (codon 31 polymorphism) of Waf-1 is also involved in this process and that there is an interaction between Waf-1 and p53 polymorphisms. To do this, Waf-1 and p53 genotypes were determined for women enrolled in a breast cancer case-control study (Caucasians, African-Americans and Latinas; 487 Waf-1 and 504 p53 genotypes were obtained). Multivariate logistic regression was used to evaluate possible associations between Waf-1 and p53 polymorphisms, race, and menopause. The primary aim was to determine whether an interaction between Waf-1 and p53-1 existed. Whereas multivariate analysis suggested associations between breast cancer and inheritance of Waf-1ser31 in African-Americans (OR, 2.32; 95% CI = 0.66-5.60; n = 37 cases and 65 controls) and Latinas (OR, 2.22; 95% CI = 0.71-6.89; n = 30 cases and 75 controls), and inheritance of p53-1-2-1 in Caucasians (OR, 3.15; 95% CI = 1.14-8.89; n = 93 cases and 187 controls), we did not see an interaction between Waf-1ser31 and p53-1-2-1. Consistent with the finding that p53-1-2-1 is a risk factor for Caucasian women was the observation of a strong interaction between race and p53 (P < 0.01).


http://cebp.aacrjournals.org/cgi/content/abstract/11/12/1684

An Arg/Pro polymorphism in codon 72 of the TP53 gene was analyzed in blood samples from 390 breast and 162 colorectal cancer patients previously investigated for TP53 mutations in their tumors. Among the breast cancer cases, 228 were homozygous for the Arg72 allele, of which, 65 (28.5%) also had a TP53 mutation in their tumors. In contrast, of 26 cases that were homozygous for the Pro72 allele, only 1 case (3.8%) had a TP53 mutation in the tumor (P = 0.004). Cloning the TP53 gene from tumor DNA followed by sequencing was performed in 14 heterozygotes with tumor mutation, and 9 of the mutations resided on the Arg72 allele. Among the colorectal cancer cases, no difference in mutation frequency was seen between the two different homozygotes, 40 TP53 mutations in 97 Arg72 homozygous cases (41.2%) versus 7 in 16 Pro72 homozygous cases (43.8%). These results suggest a selective growth advantage for cells carrying a type of TP53 mutation seen in breast carcinomas when the mutation resides on an Arg72 allele.


http://cebp.aacrjournals.org/cgi/content/abstract/14/1/108

Introduction: The oral squamous cell carcinoma (OSCC) is the sixth most common malignant tumor worldwide. No significant better progress has been made in the treatment of OSCCs during the last decades. The heterodimeric CD97 protein is a epidermal growth factor seven-transmembrane family member and was identified as a dedifferentiation marker in thyroid carcinomas. Nothing is known about CD97 in OSCCs. Material and Methods: Employing UV-laser microdissection, CD97 and its ligand CD55 were investigated in normal oral mucosa and OSCCs (n = 78) by multiplex reverse transcription-PCR. Frozen sections were investigated by immunohistochemistry. The effects of retinoic acid and sodium butyrate on the CD97/CD55 expression in OSCC cell lines were determined by quantitative PCR, immunocytochemistry, and flow cytometry. Results: Weak CD97 transcripts were expressed in normal mucosa and normal basal epithelial cells revealed specific CD97 immunostaining. Strong CD97 transcripts were detected in pT3/T4 and G3/G4 OSCC tissues, whereas pT1/T2 and G1/G2 carcinomas revealed weak CD97 transcript levels. A weak CD97 immunostaining was observed in pT1/T2 and G1/G2 tumors. By contrast, intensive CD97 immunostaining was detected in pT3/T4 OSCCs and G3/G4
lesions. CD55 gene expression was low in normal mucosa. All OSCCs, irrespective of stage and grading, displayed strong CD55 immunostaining. Sodium butyrate and retinoic acid inhibited CD97 mRNA and protein in OSCC cell lines. Interestingly, CD55 was up-regulated by both substances. Conclusion: We identified CD97 as a novel marker of dedifferentiated OSCC. Interaction of CD97 and CD55 may facilitate adhesion of OSCC cells to surrounding surfaces that would result in metastases and bad prognosis.


http://cebp.aacrjournals.org/cgi/content/abstract/13/4/662

JC virus (JCV) is an ubiquitous human polyomavirus that frequently resides in the kidneys of healthy individuals and is excreted in the urine of a large proportion of the adult population. Polyomaviruses are associated with disease largely in immunocompromised individuals (progressive multifocal leukoencephalopathy). Colorectal cancers can show chromosome instability and it was hypothesized that JCV may account for some of this instability. We screened urine from 45 healthy donors and 233 colorectal cancer/normal tissue pairs for the presence of JCV sequences using a Taqman assay. This assay could detect 1 virus genome in 10 human genomes. In the urine samples, we found an infection rate of approximately 70%. The JCV isolates in these samples could be categorized into four JCV types (2B, 4, 7, and 8), none of which had a rearranged regulatory region. Among the colon tissues, one normal tissue (<0.5%) and none of the matched tumors tested positive for JCV. There is no evidence in these data to indicate that JCV is the cause of genetic instability in colorectal cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/815

Endogenous sex hormones play an important role in the etiology of breast cancer. Polymorphisms in genes encoding for enzymes involved in steroidogenesis may therefore play a role in breast cancer risk. Cytochrome P450c17(alph) (Cyp17) functions at key branch points in human steroidogenesis. A T\[-&gt;C\] transition (A1 and A2 allele) in the 5' untranslated region may be associated with increased expression of Cyp17. Using a case-cohort design, we studied the effects of the A2 allele on endogenous sex hormone levels and breast cancer risk within a large population-based cohort (n = 9,349) in the Netherlands (the DOM-cohort). Cyp17 genotype was determined in 335 incident postmenopausal breast cancer cases, which occurred after follow-up (median time to follow-up, 19 years) of the entire cohort, and in a random sample of 373 women (subcohort). Concentrations of estrone (E1), estradiol (E2), testosterone, 5(alpha)-androstane-3(alpha), 17(beta)-dial (3(alpha)D), and creatinine were measured in first-morning urine samples. Only among women with body mass index (BMI) < 25 kg/m2 was the A2A2 genotype associated with higher levels of E1, E2, and 3(alpha)D compared with a group of women with either the A1A1 or the A1A2 genotype (e.g., geometric means of E1 in ng/mgcreatinine: A2A2, 2.23; A1A1/A1A2, 1.47; P = 0.03). Adjusted breast cancer rate ratios for women with the A1A2 or A2A2 genotype compared with women with the A1A1 genotype were 0.96 (0.68-1.37) and 0.80 (0.47-1.35), respectively. These results did not differ between women with low and high BMI. In conclusion, this paper shows that women with low BMI and the A2A2 genotype had higher endogenous sex steroid levels compared with women with the A1A1 genotype. However, these increased sex steroid levels are not translated into an increased breast cancer risk in these women.
Multiple conflicting findings have been presented which indicate that EBV may be found in anywhere from 0% to 51% of breast carcinomas. When EBV has been found causally associated with other human cancers, its DNA and one or more of its viral products have been detected in most tumor cells of a given biopsy. To test whether EBV has such an association with breast cancer, we measured the number of viral DNA molecules per cell in matched normal and tumor biopsies from 45 patients using real-time quantitative PCR. In no case could EBV DNA consistently be detected, with either of two different probes, at levels above 0.1 molecules per cell in two sections of the tumor samples. These levels of detection match those detected in EBV-negative cell lines and therefore likely represent noise in the assays. Equally importantly, the distribution of these low signals was the same between tumors and their matched normal controls. We conclude that EBV does not contribute to the development of breast cancers as it does to epithelial cancers such as nasopharyngeal and gastric carcinomas or to Burkitt's and Hodgkin's lymphomas.


We have evaluated the use of allele-specific PCR (AS PCR) on DNA pools as a tool for screening inherited genetic variants that may be associated with risk of adult acute myeloid leukemia (AML). Two DNA pools were constructed, one of 444 AML cases, and another of 823 matched controls. The pools were validated using individual genotyping data for GSTP1 and LT(alpha) variants. Allele frequencies for variants in GSTP1 and LT(alpha) were estimated using quantitative AS PCR, and when compared to individual genotyping data, a high degree of concordance was seen. AS primer pairs were designed for nine candidate genetic variants in DNA repair and cell cycle/apoptotic regulatory genes, including Cyclin D1 [codon 870 splice site variant (A>G)]; BRCA1, P871L; ERCC2, K751Q; FAS -1377 (G>A); hMLH1 -93 (G>A) and V219I; p21, S31R; and the XRCC1 R194W and R399Q variants. For six of these assays, there was at least 95% concordance between AS PCR genotyping and an alternative approach carried out on individual samples. Furthermore, these six AS PCR assays all accurately estimated allele frequencies in the pools that had been calculated using individual genotyping data. A significant disease association was seen with AML for the -1377 variant in FAS (odds ratio 1.76, 95% confidence interval 1.26-2.44). These data suggest that quantitative AS PCR can be used as an efficient screening technique for disease associations of genetic variants in DNA pools made from case-control studies.


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Estrogen unopposed by progestins is a key factor in endometrial cancer etiology. Cytochrome P450 1B1 (CYP1B1), responsible for the 4-hydroxylation of estrogen, may be important in endometrial carcinogenesis, either as a regulator of estrogen availability or as a producer of potentially genotoxic estrogen metabolites. We investigated the association of CYP1B1 genotype and endometrial cancer risk in a population-based case-control study of postmenopausal Swedish women. We used the Expectation-Maximization algorithm to estimate the haplotype frequencies in the population and calculated odds ratios and 95% confidence intervals from conditional logistic regression models. In stratified analysis, we investigated the possible effects of CYP1B1 genotype on endometrial cancer risk in subgroups defined primarily by menopausal hormone use and also by body mass index, smoking, use of combined oral contraceptives, and family history. We genotyped 689 cases and 1,549 controls for the CYP1B1 single nucleotide polymorphisms m2, m3, and m4 and estimated the haplotype frequencies among controls to 0.086, 0.291, 0.452, and 0.169 for the CYP1B1*1, CYP1B1*2, CYP1B1*3, and CYP1B1*4 alleles, respectively. We found no evidence for an overall association between CYP1B1 genotype and endometrial cancer risk, nor was there any clear indication of gene-environment interaction.


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The population of Linxian in north central China is at high risk for gastric cardia adenocarcinoma (GCC) and esophageal squamous cell carcinoma (ESCC), and chronic inflammation may contribute to this risk. Interleukin-8 (IL8), a potent chemoattractant, has three well-characterized single nucleotide polymorphisms (SNP), one (-251) of which alters transcriptional activity. Four well-described SNPs in the two IL8 receptors, IL8RA and IL8RB, have been associated with inflammation. We conducted a case-cohort study in the Nutrition Intervention Trials (Linxian, China) to assess the association between these SNPs and incident GCC (n = 90) and ESCC (n = 131). IL8, IL8RA, and IL8RB SNPs were analyzed using a multiplex assay system, haplotypes were constructed, and risks were estimated using Cox proportional hazards models. The homozygous variants of IL8 -251 and +396 were associated with 2-fold increased relative risks for GCC, but the highest risk observed was for the AGT/AGC haplotype of IL8 -251/+396/+781 (relative risk, 4.14; 95% confidence interval, 1.31-13.1). Variation within IL8 was not associated with ESCC. Few subjects had variation at the IL8RA SNP and no significant associations were observed for IL8RB SNPs or haplotypes with either GCC or ESCC. We conclude that variation in IL8 seems to increase the risk for GCC but not ESCC in this high-risk population. These variants could confer an altered IL8 expression pattern or interact with environmental factors to increase the risk for inflammation and GCC.

A polymorphism at codon 72 of the human tumor suppressor p53 determines translation into either arginine or proline. Yet, the impact of this amino acid variability on the risk to develop malignant tumors, particularly carcinomas associated with human papilloma virus (HPV) infections, remains unresolved because of contradictory results. To address a potential correlation between the different genotypes and the manifestation of squamous cell carcinomas of the head and neck (SCCHN), we determined the p53 codon 72 in 193 healthy subjects and 122 unselected SCCHN with known HPV status. Furthermore, loss of allele-specific transcription was analyzed in p53 codon 72 heterozygous (Arg/Pro) SCCHN and correlated with HPV 16 and/or 18 E6 transcript expression. We found a moderately increased risk (odds ratio, 1.86; 95% confidence interval, 1.0-3.3) for individuals with germ line heterozygosity to develop SCC of the pharynx. On the other hand, p53 codon 72 polymorphic variants, most notably the Arg/Arg genotype, showed no association with the presence of HPV 16 and/or 18 E6 transcript. Moreover, there was no evidence for HPV-driven selection in SCCHN with allele-specific loss of transcription. Our data suggest that the p53 codon 72 polymorphism has a minor impact on the development of SCCHN.


Ras proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, including leukemia. While both human and animal studies have linked several chemical carcinogens to specific ras mutations, little data exist regarding the association of ras mutations with parental exposures and risk of childhood leukemia. Using data from a large case-control study of childhood acute lymphoblastic leukemia (ALL; age <15 years) conducted by the Children's Cancer Group, we used a case-case comparison approach to examine whether reported parental exposure to hydrocarbons at work or use of specific medications are related to ras gene mutations in the leukemia cells of children with ALL. DNA was extracted from archived bone marrow slides or cryopreserved marrow samples for 837 ALL cases. We examined mutations in K-ras and N-ras genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. We interviewed mothers and, if available, fathers by telephone to collect exposure information. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from logistic regression to examine the association of parental exposures with ras mutations. A total of 127 (15.2%) cases had ras mutations (K-ras 4.7% and N-ras 10.68%). Both maternal (OR 3.2, 95% CI 1.7-6.1) and paternal (OR 2.0, 95% CI 1.1-3.7) reported use of mind-altering drugs were associated with N-ras mutations. Paternal use of amphetamines or diet pills was associated with N-ras mutations (OR 4.1, 95% CI 1.1-15.0); no association was observed with maternal use. Maternal exposure to solvents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8) were related to K-ras mutation. Maternal ever exposure to oil and coal products before case diagnosis (OR 2.3, 95% CI 1.1-4.8) and during the postnatal period (OR 2.2, 95% CI 1.0-5.5) and paternal exposure to plastic materials before index pregnancy (OR 2.4, 95% CI 1.1-5.1) and other hydrocarbons during the postnatal period (OR 1.8, 95% CI 1.0-1.3) were associated with N-ras mutations. This study suggests that parental exposure to specific chemicals may be associated with distinct ras mutations in children who develop ALL.
Introduction: Insulin, insulin-like growth factor (IGF), and IGF binding protein (IGFBP) are involved in cell growth and proliferation and are thought to be important in the etiology of colorectal cancer. We hypothesize that genetic polymorphisms of insulin receptor substrates (IRS-1 and IRS-2), IGF-I, and IGFBP-3 alter colorectal cancer risk because of their roles in the insulin-related signaling pathway. Methods: Data from a population-based incident case-control study of 1,346 colon cancer cases and 1,544 population-based controls and 952 rectal cancer cases and 1,205 controls were used to evaluate associations. Genetic polymorphisms of four genes were investigated: an IGF1 CA repeat, the IGFBP3 -202 A > C, the IRS1 G972R, and the IRS2 G1057D. Results: Having at least one R allele (GR or RR) for IRS1 G972R was associated with an increased risk of colon cancer [odds ratio 1.4, 95% confidence interval (95% CI) 1.1-1.9]. The IRS2 G972R heterozygote GD genotype significantly reduced risk of colon cancer (odds ratio 0.8, 95% CI 0.6-0.9). Neither the IGF1 nor the IGFBP3 variants was associated independently with colon cancer, but there was an association when examined with IRS1. Individuals with an IRS1 R allele and IGF1 non-192 allele were at a 2-fold increased risk of colon cancer (95% CI 1.2-4.4). There was a 70% (95% CI 1.02-2.8) increased risk of colon cancer with an IRS1 R allele and the IGFBP3 AC or CC genotype. The IRS2 GD genotype reduced risk of colon cancer, except among those with an IRS1 R allele. No significant associations were seen in analyses of main effects or interactions of these variants and rectal cancer risk. Conclusions: Both IRS1 and IRS2 variants were associated with colon cancer risk independently. Associations were slightly stronger when polymorphisms in multiple genes were evaluated in conjunction with other genes rather than individually. These data suggest that the insulin-related pathway may be important in the etiology of colon cancer but not rectal cancer.

Introduction: Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce risk of colorectal cancer. Although inhibition of cyclooxygenase (COX)-2 is generally thought to be the relevant mechanism, aspirin-like drugs apparently are involved in other pathways and mechanisms. We explore the associations between aspirin/NSAIDs, the insulin-related pathway, and the risk of colorectal cancer. Methods: Genetic polymorphisms of five genes identified as being involved in an insulin-related pathway were genotyped using data collected in a case-control study of 1346 incident colon cancer cases and 1544 population-based controls and 952 incident rectal cancer cases and 1205 controls. Genotypes assessed were the 3’ untranslated region poly(A) and the intron 8 BsmI polymorphisms of the VDR gene, a CA repeat polymorphism of the IGF1 gene, the A/C polymorphism at nucleotide -202 of the IGFBP3, the Gly972Arg polymorphism of the IRS1 gene, and the Gly1057Asp polymorphism of the IRS2 gene. Results: Use of aspirin and NSAIDs was associated with a decreased risk of colorectal cancer, with slightly greater protection from NSAIDs than aspirin for rectal cancer. We observed a significant interaction between IRS1 genotype and aspirin/NSAIDs use and risk of colorectal cancer. Relative to the GR/RR IRS1 genotype, a protective effect from the GG IRS1 genotype was seen in those who did not use NSAIDs; use of NSAIDs was protective for all genotypes. These associations were especially strong for those diagnosed prior to age 65 (P interaction = 0.0006). We also observed a significant interaction between aspirin/NSAIDs use and the VDR gene. Having the SS or BB VDR genotypes reduced risk of colorectal cancer among non-aspirin/NSAID users; however, aspirin/NSAIDs reduced risk for all VDR genotypes. Conclusions: These data support the protective effect of aspirin and NSAIDs on colorectal cancer risk. In
addition, the observed interactions for aspirin/NSAIDs and IRS1 and VDR genotypes suggest that mechanisms other than COX-2 inhibition may be contributing to the protective effect of aspirin and NSAIDs on colorectal cancer risk.


Cigarette smoking is the main risk factor for bladder cancer, accounting for at least 50% of bladder cancer in men. Cigarette smoke is a rich source of arylamines, which are detoxified by the NAT2 enzyme and activated by the NAT1 enzyme to highly reactive species that can form bulky adducts on DNA. DNA damage from such adducts is mainly repaired by the nucleotide excision repair pathway, in which the XPD protein functions in opening the DNA helix. We hypothesized that an XPD codon 751 polymorphism (Lys-to-Gln amino acid change) could affect the repair of smoking-induced DNA damage and could be associated with bladder-cancer risk. We also hypothesized that allelic variants of the NAT1 and NAT2 genes might modify the effect of the XPD codon 751 polymorphism on smoking-associated bladder-cancer risk. We determined the XPD codon 751 genotype for 228 bladder-cancer cases and 210 controls who were frequency-matched to cases by age, sex, and ethnicity, and we used our previously published data on the NAT1 and NAT2 genotypes for these same individuals (J. A. Taylor et al., Cancer Res., 58: 3603-3610, 1998). We found a slight decrease in risk for the XPD codon 751 Gln/Gln genotype (adjusted odds ratio: 0.8; 95% confidence interval: 0.4-1.3) compared with subjects with the Lys/Lys or Lys/Gln genotypes. The analysis with smoking showed that smokers with the Lys/Lys or Lys/Gln genotypes were twice as likely to have bladder cancer than smokers with the Gln/Gln genotype (test of interaction P = 0.03). The combined presence of the NAT1/NAT2 high-risk genotype and the XPD Lys/Lys or Lys/Gln genotypes ignoring smoking had an odds ratio that was only slightly higher than expected, assuming no genotype-genotype interaction (P = 0.52). We found little evidence for a gene-gene-exposure, three-way interaction among the XPD codon 751 genotype, smoking, and the NAT1/NAT2 genotype.


DNA repair efficiency varies among individuals, with reduced repair capacity as a risk factor for various cancers. This variability could be partly explained by allelic variants for different DNA repair genes. We examined the role of a common polymorphism in the XRCC3 gene (codon 241: threonine to methionine change) and bladder cancer risk. This gene plays a role in the homologous recombination pathway, which repairs double-strand breaks. The functional consequences of the XRCC3 codon 241 polymorphism are still unknown. We hypothesized that this polymorphism could affect repair of smoking-associated DNA damage and could thereby affect bladder cancer risk. We genotyped 233 bladder cancer cases and 209 controls who had been frequency matched to cases on age, sex, and ethnicity. We observed little evidence of a positive association between subjects who carried at least one copy of the codon 241 Met allele and bladder cancer (odds ratio: 1.3; 95% confidence interval: 0.9-1.9). Among heavy smokers, individuals with the Met allele had about twice the risk of those without it; however, a test of interaction was not statistically significant (P = 0.26). Previously, we observed in these subjects an association between bladder cancer risk and allelic variants of the XRCC1 gene, which is involved in the repair of base damage and single-strand breaks. In this study, we found some
evidence for a gene-gene interaction between the XRCC1 codon 194 and XRCC3 codon 241 polymorphisms (P = 0.09) and some support for a possible gene-gene-smoking three-way interaction (P = 0.08).


http://cebp.aacrjournals.org/cgi/content/abstract/11/8/730

Lung adenocarcinoma has replaced squamous cell lung carcinoma as the most frequent histological subtype in lung cancers. However, genetic factors that affect cancer susceptibility are much less understood in adenocarcinoma than in squamous cell carcinoma. In this study, polymorphisms in five genes involved in the metabolism of carcinogens or in the repair of damaged DNA in lung cells, NQO1-Pro187Ser, GSTT1-positive/null, GSTM1-positive/null, CYP1A1-Ile462Val, and OGG1-Ser326Cys, were examined for association with lung adenocarcinoma risk in a case-control study of 198 patients and 152 control subjects. The NQO1 and GSTT1 polymorphisms were associated with lung adenocarcinoma risk with adjusted odds ratio of 2.15 for the NQO1-Pro/Pro genotype versus the Ser/Ser genotype and adjusted odds ratio of 1.61 for the GSTT1-null genotype versus the positive genotype, respectively. Furthermore, individuals with the combined genotype of NQO1-Pro/Pro and GSTT1-null showed greater risk compared with those of NQO1-Ser/Ser and GSTT1-positive. In contrast, significant association was not observed for the GSTM1, CYP1A1, and OGG1 polymorphisms with lung adenocarcinoma risk, although several studies have shown their implication in the risk for squamous cell lung carcinoma. The result indicates that the NQO1-Pro/Pro and GSTT1-null genotypes are risk factors for lung adenocarcinoma development, and that the genetic factors for susceptibility to adenocarcinoma are different from those to squamous cell carcinoma. The enhanced risk of the NQO1-Pro/Pro genotype combined with the GSTT1-null genotype was more evident in smokers than in nonsmokers. Therefore, carcinogens in tobacco smoke, which are activated by NQO1 and detoxified by GSTT1, could have a role in lung adenocarcinoma development.


http://cebp.aacrjournals.org/cgi/content/abstract/11/8/771

In large studies and under field conditions common to epidemiological research, factors outside of and inside the laboratory can introduce misclassification of genetic susceptibility markers. Few reports have been made on the accuracy of genotyping individuals using DNA extracted from frozen urine that was stored for [~]20 years. This study was performed to determine the reproducibility and accuracy of N-acetyltransferase 2 (NAT2) genotyping by RFLP analysis using DNA from stored urine. To obtain long-term frozen urine and blood samples from the same person, the databases of two large prospective studies were linked by name and date of birth. Six polymorphisms within the coding region of NAT2 were determined in 65 urine and blood samples after which, genotypes and imputed phenotypes (rapid, slow) were derived. To test reproducibility, all of the six polymorphisms were determined twice in 47 urine-blood pairs. Reproducibility of imputed phenotypes was 91.5% in urine samples and 97.9% in blood samples. To test accuracy, results for all six polymorphisms were compared between urine and blood samples. All of the {kappa}'s were at least 0.85 except one. Identical results for all six polymorphisms were seen in 78.5% of urine-blood pairs. Taking blood samples as a reference standard, rapid acetylators were classified as rapid in 97% of subjects (95% confidence interval, 90-100%), and
slow acetylators were classified as slow also in 97% of subjects (95% confidence interval, 91-100%), when using urine. This study shows that stored urine samples can be used for DNA genotyping in large cohort studies, when blood samples are not available.


http://cebp.aacrjournals.org/cgi/content/abstract/12/11/1194

Women with high androgen levels appear to be at increased risk for breast cancer. The 5-{alpha}-reductase type 2 enzyme (SRD5A2) is an important mediator of local androgen actions. The SRD5A2 gene contains a polymorphism leading to a valine to leucine change in codon 89 (V89L). The Leu allele has been associated with lower SRD5A2 activity and might be protective for breast cancer. At the same time, among breast cancer patients, the Leu allele has been associated with lower prostate-specific antigen expression, indicating poor prognosis. Within a cohort of breast cancer screening participants in the Netherlands (DOM-cohort) we examined whether the V89L polymorphism is associated with the risk and prognosis of breast cancer. We studied 295 postmenopausal breast cancer cases and a randomly selected reference group from the baseline cohort (n = 382). The genotype distribution in the reference group was: VV 52%; VL 40%; and LL 8%. Compared with women with the VV genotype, adjusted breast cancer rate ratios for women with VL and LL genotypes were 1.5 (95% confidence interval = 1.0-2.2) and 1.1 (95% confidence interval = 0.5-2.1), respectively. Compared with breast cancer patients with VV or VL genotypes, those with the LL genotype showed larger tumors (proportion with size > 2 cm is 26 versus 55%, respectively; P = 0.07), a higher frequency of positive lymph nodes (28 versus 55%, respectively; P = 0.09), and a higher tumor-node-metastasis stage (proportion with stage III/IV: 6 versus 25%, respectively; P = 0.04). The LL genotype is also associated with shorter survival than the VV and VL genotypes (P = 0.10). In conclusion, our findings do not provide evidence for an important role of the V89L polymorphism in the etiology of breast cancer. However, in breast cancer patients, the LL genotype may be associated with unfavorable prognosis.


http://cebp.aacrjournals.org/cgi/content/abstract/13/2/324

Whether antibodies to human papillomavirus (HPV) capsids, elicited by natural infection, are protective is unknown. This question was addressed in a population-based cohort of 7046 women in Costa Rica by examining the association between baseline seroreactivity to HPV-16, HPV-18, or HPV-31 virus-like particles and the risk of subsequent HPV infection at a follow-up visit 5-7 years after enrollment. Seropositivity to HPV-16, HPV-18, or HPV-31 was not associated with a statistically significant decreased risk of infection with the homologous HPV type [relative risk (RR) and [95% confidence interval (CI)], 0.74 (0.45-1.2), 1.5 (0.83-2.7), and 0.94 (0.48-1.8), respectively]. Seropositivity to HPV-16 or HPV-31 was not associated with a decreased risk of infection with HPV-16 or its genetically related types [RR (95% CI), 0.82 (0.61-1.1) and 0.93 (0.68-1.2), respectively]. Seropositivity to HPV-18 was not associated with a decreased risk of infection with HPV-18 or its genetically related types (RR 1.3; 95% CI 1.0-1.8). Thus, we did not observe immunity, although a protective effect from natural infection cannot be excluded because of the limits of available assays and study designs.
In this paper, we present evidence that alleles of several polymorphisms in the chromosomal region 19q13.2-3, encompassing the genes RAI and XPD, are associated with occurrence of basal cell carcinoma in Caucasian Americans. The association of one of these, RAI-intron1, is sufficiently strong to make mass significance unlikely (P = 0.004, \( \chi^2 \)). We interpret our combined data to indicate that a specific haplotype partly defined by the alleles of three single nucleotide polymorphisms, RAI intron1G, RAI exon6T, and XPD exon 6C, is associated with a protective gene variant in a region spanning from XPD to ERCC1.


http://cebp.aacrjournals.org/cgi/content/abstract/14/2/451

There have been few studies of the associations of genetic polymorphisms with precancerous gastric lesions. We conducted a cross-sectional study to compare the prevalences of several genetic polymorphisms in 302 subjects with mild chronic atrophic gastritis with prevalences in 606 subjects with deep intestinal metaplasia or dysplasia. This stratified random sample of 908 subjects was selected and analyzed for genetic polymorphisms from 2,628 individuals who had gastric biopsies with histopathology in 1989 in Linqu County, Shandong Province, China. In subjects with mild chronic atrophic gastritis, the frequencies of the variant (less common) alleles of CYP2E1 Rsal, CYP2E1 DraI, GSTP1, ALDH2, and ODC were, respectively, 0.156, 0.201, 0.189, 0.190, and 0.428. The frequencies of the null genotypes of GSTM1 and GSTT1 in the mild chronic atrophic gastritis group were 0.509 and 0.565, respectively. Comparing mild chronic atrophic gastritis with deep intestinal metaplasia or any degree of dysplasia, we found no statistically significant associations with any genotype from these loci for dominant, additive, or recessive inheritance models. There was no statistically significant evidence of multiplicative interactions between any pair of genotypes based on CYP2E1 Rsal, CYP2E1 DraI, GSTP1, GSTM1, or GSTT1; nor between Helicobacter pylori status and any of these five loci; nor between smoking status and GSTP1, GSTM1, or GSTT1; nor between alcohol consumption and ALDH2. Statistically significant interactions were noted between salt consumption and GSTP1 and between sour pancake consumption and CYP2E1 Rsal. There was, moreover, a statistically significant interaction (odds ratio, 1.78; 95% confidence interval, 1.03-3.08) between CYP2E1 DraI and smoking at least one cigarette per day. A positive but not statistically significant interaction was also seen between CYP2E1 Rsal and smoking status. These polymorphisms do not seem to govern progression from mild chronic atrophic gastritis to advanced precancerous gastric lesions, but the effects of smoking may be accentuated in individuals carrying variants of CYP2E1.
The CYP11A gene encodes the cholesterol side-chain cleavage enzyme (P450scc) that catalyzes the first and rate-limiting step for the biosynthesis of sex hormones. A pentanucleotide repeat [(TAAAA)n] polymorphism in the 5' of the CYP11A gene has been reported to be related to the risk of polycystic ovary syndrome, an inherited endocrine disorder characterized by hyperandrogenemia. We investigated the association of this polymorphism with breast cancer risk in a population-based case-control study conducted among Chinese women in Shanghai. Genotype assays were completed for 1015 incident breast cancer cases and 1082 community controls. Three common alleles with 4, 6, or 8 TAAAA repeats were identified in the study population. The frequency of the 8 repeat allele was more common in cases (12.6%) than controls (8.5%) (odds ratio = 1.6, 95% confidence interval = 1.3-1.9; P < 0.0001). Compared to subjects who did not carry this allele, adjusted odds ratios were 1.5 (95% confidence interval = 1.2-1.9) and 2.9 (1.3-6.7) (P for trend, <0.001), respectively, for those who carried one and two copies of this allele. This positive association was observed in both pre- and postmenopausal women and all strata defined by major breast cancer risk factors, including years of menstruation, body mass index, and waist-to-hip ratio. The results from this study indicate that the TAAAA repeat polymorphism near the promoter region of the CYP11A gene may be an important susceptibility factor for breast cancer risk.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/830

Most esophageal adenocarcinomas arise within Barrett's esophagus but the cause of this increasingly prevalent condition remains unknown. Early detection improves survival and discriminant screening markers for Barrett's esophagus and cancer are needed. This study was designed to explore the natural history of eyes absent 4 (EYA4) gene methylation in the neoplastic progression of Barrett's esophagus and to evaluate methylated EYA4 as a candidate marker. Aberrant promoter methylation of EYA4 was studied by methylation-specific PCR using bisulfite-treated DNA from esophageal adenocarcinomas, Barrett's esophagus, and normal epithelia, and then confirmed by sequencing. Eight cancer cell lines were treated with the demethylation agent 5-aza-2'-deoxycytidine, and EYA4 mRNA expression with and without treatment was quantified by real-time reverse-transcription PCR. EYA4 hypermethylation was detected in 83% (33 of 40) of esophageal adenocarcinomas and 77% (27 of 35) of Barrett's tissues, but only in 3% (2 of 58) of normal esophageal and gastric mucosa samples (P < 0.001). The unmethylated cancer cell lines had much higher EYA4 mRNA expression than the methylated cancer cell lines. Demethylation caused by 5-aza-2'-deoxycytidine increased the mRNA expression level by a median of 3.2-fold in methylated cells, but its effect on unmethylated cells was negligible. Results indicate that aberrant promoter methylation of EYA4 is very common during tumorigenesis in Barrett's esophagus, occurs in early metaplasia, seems to be an important mechanism of down-regulating EYA4 expression, and represents an intriguing candidate marker for Barrett's metaplasia and esophageal cancer.

To evaluate the mechanism for the biological activity of a natural polysaccharide isolated from the mucus of the loach, Misgurnus anguillicudatus (MAP), the immunomodulatory of MAP was investigated by the methods of molecular biology and cellular biology. The results showed that MAP enhanced proliferation of T lymphocyte, IL-2 expression of Th1 cells, and IL-4 expression of Th2 cells. Time dependence of the secretion of cytokines showed that Th1 cell was the primary cellular target affected by MAP on T lymphocyte. However, MAP did not increase directly the proliferation of B cells and enhanced less IgM antibody production. Moreover, MAP improved the viability of peritoneal macrophages, stimulated TNF-\(\alpha\) and IL-6 production and induced the inducible nitric oxide synthase (iNOS) transcription in macrophages. In addition, MAP exerted its immunomodulating activity at an optimal dose of 30 \([\mu\text{g/ml}]\). At this concentration, MAP promoted farthest proliferation of spleen lymphocyte and macrophages. Consequently, MAP enhanced the immune system functions. In conclusion, the biological activity of the loach, which was as traditional Chinese medicine in folk remedies for the treatments of hepatitis, osteomyelitis, carbuncles, inflammations and cancers, as well as for the restoration to health in debilities by various pathogens and aging, may mainly result from MAP selectively activating T cells and macrophages and stimulating secretion of some cytokines.

Carbohydrate Research


The cDNA of Chinese hamster ovary (CHO) cell cytosolic sialidase was amplified by RT-PCR and cloned into the pGEX-2T plasmid vector encoding for glutathione S-transferase (GST). Screening revealed transformed Escherichia coli clones with the constructed plasmid encoding the CHO cell sialidase sequence. After isopropyl-\(\beta\)-thiogalactopyranoside (IPTG) induction, SDS-PAGE of the total protein extracts revealed a new protein of about 70 kDa, correlating with the molecular weight of a fusion protein composed of the GST (26 kDa) and the cloned cytosolic CHO cell sialidase (43 kDa). A soluble fusion protein was purified from sonified E. coli homogenates by one-step affinity chromatography on Glutathione Sepharose 4B, which showed sialidase activity towards 4-methyl-umbelliferyl-\(\alpha\)-N-acetylneuraminic acid (MUF-Neu5Ac) substrate. Induction of cells with 0.1, 0.5, and 1.0 mM IPTG revealed highest total protein amounts after induction with 1.0 mM IPTG, but highest specific activity for affinity chromatography purified eluates from cultures induced with 0.1 mM IPTG. Therefore, large scale production was performed by inducing cells during exponential growth in a 25 L bioreactor for 3 h with 0.1 mM IPTG after chilling the cell suspension to 25[^\text{\[deg\]C}]. The amount of 26.46 mg of 40-fold purified GST-sialidase with a specific activity of 0.999 U/mg protein was obtained from crude protein extracts by one-step affinity chromatography. 2-Deoxy-2,3-dehydro-N-acetylneuraminic acid (Neu5Ac2en) and Neu5Ac were competitive inhibitors for the sialidase, the former being the more effective one using MUF-Neu5Ac as the substrate. The cytosolic sialidase is capable of desialylating a wide spectrum of different types of gangliosides using a thin-layer chromatography overlay kinetic assay without detergents. This is the subject of the accompanying paper (Muthing, J.; Burg, M. Carbohydr. Res. 2001, 330, 347-356).

http://carcin.oupjournals.org/cgi/content/abstract/25/5/749

Mutations of the N- and K-ras genes occur in ~15-30% of acute myeloid leukaemia patients. The role of the oncogenic ras in leukaemogenesis remains unclear. Few studies have revealed that mutations in the ras oncogene family are more probably found in acute myeloid leukaemia patients with previous exposure to toxic agents. A case-case study was conducted in the areas of Florence and Turin, Italy, to investigate whether the presence of N- and K-ras mutations in acute myeloid leukaemia patients was related to a higher frequency of exposure to chemicals. During a 3-year period, 111 acute myeloid leukaemia patients were enrolled. All the patients were interviewed using a semi-structured questionnaire collecting data on residential history, occupation, personal habits and pathological history. The presence of N- and K-ras mutations was analysed by amplification and synthetic oligonucleotide probes and by the so-called polymerase chain reaction amplification for specific alleles technique. A total of 34 (30.6%) patients were found to harbour ras mutations in N-ras and/or K-ras. Fourteen patients (12.6%) had a single ras mutation and 20 patients (18%) had two ras mutations. A positive association between a priori at risk jobs and ras mutations was found, based on nine exposed cases; the odds ratio, adjusted by age, sex and previous X-ray and/or chemotherapy was 2.8 (95% confidence intervals: 0.9-9.0). When considering only subjects with two ras mutations the odds ratio was 4.8 (95% confidence intervals: 1.2-18.8). The odds ratio for a previous X-ray and/or chemotherapy was 16.2 (95% confidence intervals: 1.8-755.9); when only subjects with two ras mutations were considered, the odds ratio was 26.1 (95% confidence intervals: 2.5-1248.9). In conclusion, our data suggest that ras oncogene mutations might identify a group of leukaemia in people with previous X-ray/chemotherapy or with exposure to chemical agents in the work environment.


http://carcin.oupjournals.org/cgi/content/abstract/23/8/1399

Glucoraphanin in Brassica vegetables breaks down to either sulforaphane or sulforaphane nitrile depending on the conditions, and sulforaphane can be further conjugated with glutathione. Using a high-throughput microtitre plate assay and TaqMan real time quantitative RT-PCR to measure mRNA, we show that sulforaphane and its glutathione conjugate, but not the nitrile, increased significantly (P < 0.05) both UGT1A1 and GSTA1 mRNA levels in HepG2 and HT29 cells. These changes were accompanied by an increase in UGT1A1 protein, as assessed by immunoblotting, and a 2-8-fold increase in bilirubin glucuronidation. When treated together, the nitrile derivative did not affect sulforaphane induction. The induction of UGT1A1 and GSTA1 mRNA by sulforaphane was time and concentration dependent. The results show a functional induction of glucuronidation by sulforaphane but not sulforaphane nitrile, and show that the pathway of metabolism of glucosinolates in Brassica vegetables is important in determining the resulting
biological and anticarcinogenic activities.


http://carcin.oupjournals.org/cgi/content/abstract/26/2/381

The NAD(P)H:quinone oxidoreductase 1 gene, NQO1, contains a C to T transition at amino acid codon 187, which results in very low enzymatic activity. Previous studies of the association between NQO1 genotype and lung cancer have had mixed findings. This population-based case control study examines the association between NQO1 genotype and lung cancer in the largest sample of never smokers (<100 cigarettes, lifetime) to date. Cases (n = 161) were identified through the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program, and 5-year age- and race-matched population-based controls (n = 173) were identified using random digit dialing. Allele frequencies of C and T, respectively, were 0.79 and 0.21 in Caucasians, and 0.84 and 0.16 in African Americans. Among those diagnosed aged \( \geq \)50 years, C/T and T/T genotyped individuals had 0.48 times lower lung cancer risk than individuals with C/C genotype (95% CI: 0.27-0.87). There was a non-significant suggestion of a protective effect associated with the T allele among those with a history of environmental tobacco smoke exposure (OR = 0.57, 95% CI: 0.32-1.03) but not among those without (OR = 0.98, 95% CI: 0.41-2.38). Sex, race, family history of lung cancer and histologic type did not modify the effect of NQO1 genotype on lung cancer risk. The observed risk reductions may be attributable to the greatly reduced procarcinogen activating of NAD(P)H:quinone oxidoreductase 1 in individuals with at least one copy of the T allele.


http://carcin.oupjournals.org/cgi/content/abstract/25/7/1277

Temporal- and dose-dependent changes in hepatic gene expression were examined in immature ovariectomized C57BL/6 mice gavaged with ethynyl estradiol (EE), an orally active estrogen. For temporal analysis, mice were gavaged every 24 h for 3 days with 100 \( \mu \)g/kg EE or vehicle and liver samples were collected at 2, 4, 8, 12, 24 and 72 h. Gene expression was monitored using custom cDNA microarrays containing 3067 genes/ESTs of which 393 exhibited a change at one or more time points. Functional gene annotation extracted from public databases associated temporal gene expression changes with growth and proliferation, cytoskeletal and extracellular matrix responses, microtubule-based processes, oxidative metabolism and stress, and lipid metabolism and transport. In the dose-response study, hepatic samples were collected 24 h following treatment with 0, 0.1, 1, 10, 100 or 250 \( \mu \)g/kg EE. Thirty-nine of the 79 genes identified as differentially regulated at 24 h in the time course study exhibited a dose-response relationship with an average ED50 value of 47 \( \pm \) 3.5 \( \mu \)g/kg. Comparative analysis indicated that many of the identified temporal and dose-dependent hepatic responses are similar to EE-induced uterine responses reported in the literature and in a companion study using the same animals. Results from these studies confirm that the liver is a highly estrogen responsive tissue that exhibits a number of common responses shared with the uterus as well as distinct estrogen-mediated profiles. These data will further aid in the elucidation of the mechanisms of action of estrogens in the liver as well as in other classical and non-classical estrogen responsive tissues.

http://carcin.oupjournals.org/cgi/content/abstract/26/3/525

Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are important in regulating the development, growth and differentiation of cells and have inhibitory effects on non-small cell lung cancer (NSCLC) cell growth. A comprehensive analysis of all RAR and RXR subtypes mRNA expression in a large series of patients with NSCLC and their role in the development and progression of this disease is lacking. Using a quantitative real-time RT-PCR method, we analyzed the mRNA expression of all retinoid receptor subtypes in tumor and matching normal-appearing tissues of 88 patients with NSCLC. Gene expression in tumor tissues was detected with the following frequencies: RAR\(\alpha\) 100%, RAR\(\beta\) 94%, RAR\(\gamma\) 94%, RXR\(\alpha\) 100%, RXR\(\beta\) 100% and RXR\(\gamma\) 92%. Levels of mRNA expression in tumor tissues compared with matching normal-appearing tissue were equal or reduced with the following frequencies: RAR\(\alpha\) 76.1%, RAR\(\beta\) 59.1%, RAR\(\gamma\) 39.8%, RXR\(\alpha\) 67.1%, RXR\(\beta\) 54.5% and RXR\(\gamma\) 88.6%, and were significantly associated with any one other subtype. The probability of survival was significantly different among patients with low gene expression in no or any two subtypes, any three or four subtypes or any five or six subtypes (P = 0.004, log rank test). Multivariate analysis confirmed low gene expression status as a significant independent unfavorable prognostic factor (P = 0.015). Our results show that decreased expression of all RAR and RXR receptor subtypes is a frequent event in NSCLC. Widely co-regulated down-regulation of expression of all retinoid subclasses suggests a fundamental dysregulation of the retinoid pathway in this cancer. Quantitation of RAR and RXR mRNA expression levels in tumor tissue is a candidate prognostic marker and surrogate biomarker for chemopreventive trials in NSCLC.


http://carcin.oupjournals.org/cgi/content/abstract/23/5/727

Inherited mutations of Patched (PTCH) in the nevoid basal cell carcinoma syndrome (NBCCS) lead to several developmental defects and contribute to tumor formation in a variety of tissues. PTCH mutations have been also identified in sporadic tumors associated with NBCCS including basal cell carcinoma (BCC) and medulloblastoma. Mice heterozygous for Ptc recapitulate the typical developmental symptoms of NBCCS and develop rhabdomyosarcoma (RMS) and medulloblastoma. PTCH is assumed to act as a tumor suppressor gene although inactivation of both alleles has been demonstrated only in a fraction of tumors. We have investigated the status of Ptc in RMS of heterozygous Ptc neo67/+ mice. Although the wild-type Ptc allele was retained in tumor tissue, the high levels of Ptc mRNA in these tumors result from overexpression of the mutant Ptc transcript. Our results suggest that the wild-type Ptc allele might be selectively silenced in RMS tissue or, alternatively, that haploinsufficiency of Ptc is sufficient to promote RMS formation in mice.

Betel quid (BQ) chewing, a popular habit in numerous Asian countries including India and Taiwan, has a strong correlation with an increased risk of oral squamous cell carcinoma (OSCC). While substantial efforts have been made to test the cytotoxic, genotoxic and mutagenic effects of BQ extract and its components, the disease mechanisms underlying BQ-induced oral carcinogenesis remain obscure. Here, we show that a neuronal protein, microtubule-associated protein 2 (MAP2), was induced by BQ extract in cultured normal human oral keratinocytes (NHOKs). Subsequent analyses demonstrated that such induction was more eminent and consistent in the high-molecular-weight isoform of MAP2 (hmw-MAP2) than that in its low-molecular-weight counterpart (lmw-MAP2). Furthermore, we analyzed expression of hmw-MAP2 protein in 88 oral specimens consisting of clinicopathologically pre-malignant (leukoplakia) and malignant (OSCC) lesions, along with their adjacent normal mucosa. Immunohistochemistry revealed that, with the exposure to BQ, the hmw-MAP2 was over-expressed in 41.2% (7/17) of OSCC, 11.2% (1/9) of leukoplakia and none (0/19) of normal mucosa. In contrast, expression of the hmw-MAP2 was barely detected in BQ-free OSCC. These results suggest a significant correlation between expression of the hmw-MAP2 and BQ-associated progression of oral carcinogenesis (P = 0.0046). Interestingly, the hmw-MAP2 was found to preferentially express in histopathologically less differentiated OSCC (P = 0.014); the percentages of positive staining in poorly, moderately and well differentiated OSCC were 62.5, 21.4 and 7.1%, respectively. However, BQ chewing appeared to have marginal correlation with such propensity. Finally, we show that the majority of hmw-MAP2-positive poorly differentiated lesions were also histopathologically invasive. Taken together, these findings suggest the possibility that the hmw-MAP2 may be a diagnostic marker for BQ-chewing lesions and a potential therapeutic target. To our knowledge, this study has provided the first clinical implication that closely links a cytoskeletal protein to BQ-associated oral cancer.


A rat surgical esophageal adenocarcinoma (EAC) model induced by esophagogastrroduodenal anastomosis was recently established in our laboratory. This model mimics mixed reflux of gastric and duodenal contents in human patients and produces EAC without treatment with any carcinogen. We compared the protein expression pattern between rat EAC and normal tissues by 2-dimensional protein gel electrophoresis. The overexpressed protein spots of the tumor sample were cut out and analyzed by matrix-assisted laser desorption/ionization mass spectrometry. Several stress proteins (Grp94, Grp78, calnexin, Hsp90(b) and ER61) were identified by this method. Western blotting and RT-PCR further confirmed overexpression of Grp94 in rat EAC. Immunohistochemical staining also revealed expression of Grp94 in the epithelial cells of columnar lined esophagus and EAC. Similar to the rat model, well-differentiated human EAC and gastric cardia adenocarcinomas were also found to overexpress Grp94, but esophageal squamous cell carcinomas did not. We also characterized apoptosis, cell proliferation and oxidative DNA damage in the rat tissues. Since Grp94 is known to inhibit apoptosis by maintaining intracellular Ca2+ homeostasis, our data suggest a possible correlation between oxidative stress, Grp94 overexpression and apoptosis regulation in esophageal adenocarcinogenesis.

Polymorphisms in GSTM1, GSTT1 and GSTP1 genes in humans are associated with the reduction of enzymatic activity toward several substrates, including those in tobacco smoke. To investigate the potential role these polymorphisms have, as modulators of early-onset lung cancer risk, a population-based case-control study involving early-onset lung cancer cases was performed. Biological samples were available for 350 individuals diagnosed <50 years of age identified from the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and 410 cases of age, race and sex-matched controls ascertained through random digit dialing. African Americans carrying at least one G allele at the GSTP1 locus were 2.9-fold more likely to have lung cancer compared with African Americans without a G allele after adjustment for age, sex, pack years of smoking and history of lung cancer in a first-degree relative (95% CI 1.29-6.20). African Americans with either one or two risk genotypes at the GSTM1 and GSTP1 loci were at increased risk of having lung cancer compared with those having fully functional GSTM1 and GSTP1 genes (OR = 2.8, 95% CI 1.1-7.2 and OR = 4.0, 95% CI 1.3-12.2, respectively). No significant single gene associations between GSTM1, GSTT1 or GSTP1 and early-onset lung cancer were identified in Caucasians, after adjusting for age, sex, pack years and family history of lung cancer. However, our results suggest that specific combinations of glutathione S-transferase polymorphisms increase the risk of early-onset of lung cancer. Joint analysis of these genotypes may identify individuals who are at a higher risk of developing early-onset lung cancer with a greater certainty than single gene studies.


The stearoyl-CoA desaturase 1 (Scd1) gene is involved in the synthesis and regulation of unsaturated fatty acids. Its expression is increased by several treatments/conditions that are associated with hepatocarcinogenesis (peroxisome proliferators, iron overload, dichloroacetic acid). We found that the Scd1 gene is differentially expressed, showing >10-fold higher mRNA levels in the normal liver tissue of C3H/He mice, which are genetically susceptible to hepatocarcinogenesis, than of BALB/c mice, which are resistant. Similarly, Scd1 mRNA expression was ~4-fold higher in the normal liver of F344 rats, which are susceptible to hepatocarcinogenesis, than in Brown Norway (BN) rats, which are resistant. The chromosomal location of the Scd1 locus, both in mice and rats, excludes Scd1 candidacy as a hepatocellular tumor-modifier gene, as the Scd1 locus did not show allele-specific effects in a BALB/cxC3H/He intercross or in a BNxF344 backcross and intercross. No Scd1 coding polymorphisms were detected in the mouse and the rat strains showing elevated Scd1 expression. These results suggest that the Scd1 gene represents a downstream target of hepatocellular tumor-modifier loci in two rodent species.

We reported previously that subjects homozygous for the cytochrome P450 2A6 (CYP2A6) *4 have a lower risk of lung cancer. The purpose of this study was to clarify whether or not the alterations of smoking behavior and risk for lung cancer could be found in subjects possessing novel CYP2A6 variants discovered recently. An epidemiological study was performed with 1094 cases and 611 controls in male Japanese smokers. It was found that the amounts of daily cigarette consumption in subjects who harbored CYP2A6*4/*7, *4/*10, *7/*7, *7/*9 and *4/*4 genotypes were significantly less than those in subjects carrying the *1/*1 genotype (P < 0.01). Even after adjustment with cigarette consumption, the adjusted odds ratios (ORs) for lung cancer were significantly lower in subjects who harbored CYP2A6*1/*4, *1/*7, *1/*9, *1/*10, *4/*4, *4/*7, *4/*9, *7/*7 and *7/*9 genotypes than those who possessed the *1/*1 genotype (P < 0.05). When participants were classified into four groups according to the CYP2A6 genotypes, group 1 (*1/*1), group 2 (heterozygotes for the *1 and a variant allele), group 3 (heterozygotes and homozygotes for variant alleles except for *4/*4) and group 4 (*4/*4), lung cancer risk was found to be less in subjects with the variant of CYP2A6 alleles {group 2, OR of 0.59 [95% confidence interval (CI), 0.44-0.79]; group 3, OR of 0.52 (95% CI, 0.37-0.72); group 4, OR of 0.30 (95% CI, 0.16-0.57)}. The reduced risk for lung cancer was seen more clearly in heavy smokers than in light smokers. Additional stratification analysis showed that the ORs for squamous cell carcinoma (OR of 0.07) and small cell carcinoma (OR of 0.10) were lower than that of adenocarcinoma (OR of 0.39) in group 4. These results suggest that the CYP2A6 is one of the principal determinants affecting not only smoking behavior but also susceptibility to tobacco-related lung cancer.


http://carcin.oupjournals.org/cgi/content/abstract/24/3/435

Mutational activation of (beta)-catenin and cyclin D1 over-expression are a frequent change in mouse hepatic tumors. Although activated (beta)-catenin may bind to T cell factor (TCF) family members and transcriptionally activate the cyclin D1 gene, either (beta)-catenin or cyclin D1 may be activated by various pathways independently of (beta)-catenin mutations. In this study, we investigated (beta)-catenin activation and mutations, cyclin D1 expression, H-ras mutations and phosphorylation of extracellular signal regulated protein kinases 1/2 (ERK1/2), Akt and glycogen synthetase kinase 3(beta) (GSK3(beta)) in mouse hepatic carcinogenesis. Nuclear/cytoplasmic staining of (beta)-catenin, a sign of (beta)-catenin activation, was frequently observed in association with the high nuclear cyclin D1 labeling index in the hepatic tumors at the late stage of carcinogenesis. The (beta)-catenin activation was further suggested by the fact that all hepatocellular carcinoma (HCC) cell lines examined showed the nuclear (beta)-catenin/TCF4 complex together with cyclin D1 over-expression. However, the fact that only 31.8% (7/22) of the lesions with the nuclear/cytoplasmic (beta)-catenin staining showed (beta)-catenin mutations indicated that (beta)-catenin was activated not only by its own mutations but also by other reason(s). On the other hand, there was no correlation between the (beta)-catenin/cyclin D1 activation and the H-ras mutations or phosphorylation of Akt, GSK3(beta) and ERK1/2, although GSK3(beta) was frequently over-expressed in the tumors. These results indicate that, although (beta)-catenin and cyclin D1 activation are well correlated, the Akt/GSK3(beta) and ras/ERK1/2 pathways may not play a major role in the (beta)-catenin/cyclin D1 activation.


http://carcin.oupjournals.org/cgi/content/abstract/25/12/2379
It has been shown that the matrix metalloproteinase (MMP)-1 promoter polymorphism 1G/2G is associated with an increased risk of developing various cancers including renal cell carcinoma (RCC), and is in linkage disequilibrium (LD) with the MMP-3 promoter polymorphism 5A/6A. These two genes are localized in 11q22 adjacent to each other. However, the relationship between the MMP-3 5A/6A polymorphism and susceptibility to cancer remains ambiguous. In this study, we genotyped eight polymorphisms in the region containing the MMP-1 and MMP-3 genes in 177 healthy subjects, and explored the relationships between RCC and these polymorphisms or haplotypes in 156 RCC cases and 230 age- and gender-matched controls. All the subjects studied were of Japanese descent. There were three polymorphisms that showed stronger LD with the MMP-1 1G/2G promoter variant than with the MMP-3 5A/6A promoter variant. One of these three polymorphisms was present in exon 2 of the MMP-3 gene and caused an amino acid change, Glu45Lys (G/A). When the genotype distribution of Glu45Lys was compared between RCC patients and controls, the frequency of the G/G genotype was significantly higher in the patients [age- and gender-adjusted odds ratio (OR) = 1.81, 95% confidence interval (CI) = 1.20-2.74]. A significant increase in the frequency of the 2G/2G genotype of the MMP-1 1G/2G polymorphism was also observed in the patients (age- and gender-adjusted OR = 1.86, CI = 1.23-2.82), whereas there was no significant difference for the MMP-3 5A/6A polymorphism. As expected based on these genotype-level results, the frequency of the 2G-G haplotype of MMP-1 1G/2G and MMP-3 Glu45Lys (G/A) polymorphisms was significantly higher in the patients than in the controls (crude OR = 1.95, CI = 1.31-2.91). These findings suggest that this haplotype of MMP-1 and MMP-3 variants may be associated with the risk of developing RCC.


http://carcin.oupjournals.org/cgi/content/abstract/24/4/757

We hypothesized that the mouse liver tumor response to non-genotoxic carcinogens would involve some common early gene and protein expression changes that could ultimately be used to predict chemical hepatocarcinogenesis. In order to identify a panel of genes to test, we analyzed global differences in gene and protein expression in livers from B6C3F1 mice following dietary treatment with two rodent carcinogens, the benzodiazepine anti-anxiety drug oxazepam (2500 p.p.m.) and the hypolipidemic agent Wyeth (Wy)-14,643 (500 p.p.m.) compared with livers from untreated mice. Male mice were exposed for 2 weeks and 1, 3 or 6 months to oxazepam or Wy-14,643 in an age-matched study design. By histopathological evaluation, no liver preneoplastic foci or tumors were detected at 6 months in treated or control groups. By cDNA microarray analysis [NIEHS Mouse Chip (8700 genes); n = 3 individual livers/group, four hybridizations/sample], expression of 36 genes or 220 genes were changed relative to control livers following 6 months of oxazepam or Wy-14,643 treatment, respectively. To obtain a more comprehensive picture of gene/protein expression changes, we also conducted a proteomics study by 2D-gel electrophoresis followed by matrix assisted laser desorption/ionization-mass spectrometry on cytoplasmic, nuclear, and microsomal subcellular fractions of the same liver samples utilized for the cDNA microarray analysis. Real-time PCR, western blot analysis and immunohistochemistry were utilized for validation and to expand the results to other time points. Cyp2b20, growth arrest- and damage-inducible gene (beta) (Gadd45(beta)), tumor necrosis factor (alpha)-induced protein 2 and insulin-like growth factor binding protein 1 (Igfbp5) genes and proteins were upregulated by oxazepam, and Cyp2b20, Cyclin D1, proliferating cell nuclear antigen, Igfbp5, Gadd45(beta) and cell death-inducing DNA fragmentation factor (alpha) subunit-like effector A exhibited higher expression after Wy-14,643 treatment. Most of these genes/proteins were also deregulated at 2 weeks. There appeared to be more distinct than common changes in the expression of carcinogenesis-related genes/proteins between the two compounds, suggesting that the major carcinogenic pathways are different for these compounds and may be distinct for different chemical classes.

http://carcin.oupjournals.org/cgi/content/abstract/23/10/1729

Human beings are exposed to a multitude of carcinogens in their environment, and most cancers are considered to be chemically induced. Here we examined differences in genetic alterations in rat forestomach tumors induced by repeated exposure to a genotoxic carcinogen, N-methyl-N'nitro-N-nitrosoguanidine (MNNG) or N-methylnitrosourethane (MNUR), and chronic treatment with a non-genotoxic carcinogen, butylated hydroxyanisole (BHA) or caffeic acid (CA). A total of 132, 6-week-old male F344 rats were employed. Forty rats were treated with MNNG by intragastric administration at a dose of 20 mg/kg body wt once a week for 32 weeks, and 20 rats received 20 p.p.m. MNUR in their drinking water for 48 weeks. Further groups of 20 animals were administered 2% BHA or 2% CA in the diet for 104 weeks. The remaining rats were maintained without any supplement as controls. Multiple forestomach tumors were observed in all rats of the MNNG-, MNUR-, BHA- and CA-treated groups. Histopathologically, MNUR- and CA-treated groups showed almost the same pattern. On polymerase chain reaction-single strand conformation polymorphism analysis, H-ras and p53 gene mutations were observed at high and relatively low frequencies, respectively, in forestomach tumors induced by MNNG and MNUR. Most H-ras gene mutations were G[-&gt;A transitions in codons 7 and 12 of exon 1. On the other hand, forestomach tumors due to the non-genotoxic carcinogens, BHA and CA, had almost no mutations of the H-ras and p53 genes. Moreover, relative overexpression of cyclin D1 and p53 was detected in forestomach tumors induced by the genotoxic carcinogens, while their non-genotoxic counterparts had a tendency to show low expression of those molecules. Mutations of the {beta}-catenin gene were not detected in any group. The present study demonstrates that rat forestomach tumors induced by genotoxic and non-genotoxic carcinogens have different underlying genetic alterations, even if their pathological features are similar.


http://carcin.oupjournals.org/cgi/content/abstract/25/3/309

The von Hippel-Lindau (VHL) tumor suppressor gene plays a prominent role in the development of renal cell carcinoma (RCC) in humans. VHL functions as a ubiquitin E3 ligase, controlling the stability of hypoxia inducible factor (HIF) and tumor angiogenesis. Alterations in this tumor suppressor gene are rarely observed in spontaneous or chemically induced RCC that arise in conventional strains of rodents and Vhl knockout mice (Vhl+/-) do not develop spontaneous RCC. We tested whether Vhl knockout mice exhibited increased susceptibility to renal carcinogenesis using the well-characterized renal carcinogen streptozotocin. No differences were observed between wild-type and Vhl+/- animals in the frequency or type of renal lesions induced by 50-200 mg/kg streptozotocin. Carcinogen-induced RCC that developed in Vhl heterozygotes and wild-type mice did not contain mutations in the wild-type Vhl, as determined by direct sequencing of the primary tumors. While Vhl+/- mice exhibited no increase in renal lesions in response to streptozotocin, heterozygous animals did develop vascular proliferative lesions of the liver, uterus, ovary, spleen and heart. These lesions, ranging from angiectasis to hemangiosarcoma, were most prominent in the livers of Vhl+/- mice, where they were found in high incidence and high multiplicity. Wild-type mice developed a low-frequency of liver angiectasis (7-15%) only at the highest doses of carcinogen used (150 and 200 mg/kg, respectively) while Vhl+/- mice exhibited angiectasis, hemangioma and hemangiosarcomas with a frequency ranging from 19 to 46% at 50-200 mg/kg streptozotocin. Untreated Vhl+/- mice had a spontaneous incidence of hepatic vascular lesions of 21%. Furthermore, vascular lesions of the uterus, ovary, spleen and
heart were observed only in Vhl+/- mice, with an incidence of (5-28%). Taken together, the data indicate that heterozygosity at the Vhl locus predisposes mice to a vascular phenotype ranging from angiectasis to hemangiosarcoma, consistent with the ability of this tumor suppressor gene to control the stability of HIF and regulate key proteins that participate in angiogenesis.


http://carcin.oupjournals.org/cgi/content/abstract/23/8/1281

Bile acids have been suggested to play an important role in the etiology of colon and gastric cancer after gastrectomy, but the molecular biology of these effects is poorly understood. We evaluated the effect of different bile acids on human gastric and colon carcinoma cells and identified genes by RNA arbitrarily primed PCR for differential display that are modulated following treatment with hydrophobic bile acids. Thioredoxin reductase (TR) mRNA was upregulated after treatment with taurochenodeoxycholic acid (TCDCA) in St 23132 cells. This raised the question whether deoxycholic acid (DCA) would have regulative effects on TR in HT-29 cells. After an incubation time of 6 h with DCA, TR mRNA expression was increased up to threefold. Ursodeoxycholic acid had no influence on TR mRNA expression. The upregulation of TR after DCA incubation was almost identical to incubation with 12-O-tetradecanoylphorbol-13-acetate. This implies that hydrophobic bile acids mediate oxidative stress in gastrointestinal cancer cells, which was confirmed by measurement of oxidative burst after treatment with DCA. The results suggest that hydrophobic bile acids induce oxidative stress in gastrointestinal cancer resulting in a compensatory upregulation of TR mRNA, one of the key components in the complex anti-oxidant defense system within eukaryotic cells. The activation of at least parts of the redox signaling system is potentially related to the cytotoxicity and the stimulation of the cell death machinery induced by toxic bile acids.


http://carcin.oupjournals.org/cgi/content/abstract/23/11/1885

The maternally expressed H19 gene is transcribed as an untranslated RNA that serves as a riboregulator. We have previously reported that this transcript accumulates in epithelial cells in ~10% of breast cancers. To gain further insight on how the overexpression of the H19 gene affects the phenotype of human breast epithelial cells, we investigated the oncogenic potential of RNA that was abundantly expressed from MDA-MB-231 breast cancer cells stably transfected with the genomic sequence of the human H19 gene. The amount of H19 RNA did not affect cell proliferation capacity, timing of cell cycle phases or anchorage-dependent ability of H19-recombined clones in vitro. But in anchorage-independent growth assays the H19-recombined cells formed more and larger colonies in soft-agar versus control cells. To explore this phenotypic change, we analysed tumour development after subcutaneous injection of H19-recombined cells into scid mice. Results showed that H19 overexpression promotes tumour progression. These data support the hypothesis that an overload of H19 transcript is associated with cells exhibiting higher tumorigenic phenotypes and therefore we conclude that the H19 gene has oncogenic properties in breast epithelial cells.

The pattern of somatic mutations in TP53 is distinct for particular cancers and carcinogenic exposures, providing clues to disease etiology, e.g., G:C[&gt;]T:A mutations in TP53 are more frequently observed in smoking-associated lung cancers. In order to investigate possible causes and mechanisms of lung cancer susceptibility differences, the TP53 gene was sequenced in a case-only study of lung cancers (206 men and 103 women). Our primary hypothesis was that the TP53 mutation spectrum is influenced by polymorphisms in genes involved in DNA repair and apoptosis. We observed a TP53 mutation frequency in exons 5-8 of 25%. Functional polymorphisms in XPD (Asp312Asn, rs1799793 and Lys751Gln, rs1052559), a protein required for nucleotide excision repair and with roles in p53-mediated apoptosis, were modestly associated with G:C[&gt;]T:A mutations in TP53 in lung tumors [Asp/Asn312 + Asn/Asn312 and/or Lys/Gln751 + Gln/Gln751 versus Asp/Asp312 + Lys/Lys751; odds ratio (OR) 2.73, 95% confidence interval (CI) 0.98-7.61], consistent with the role of this protein in repair of bulky carcinogen-DNA adducts. In addition, a TP53 polymorphism (Arg72Pro, rs1042522) with a known role in the efficiency of apoptosis was also associated with the presence of a TP53 mutation (Pro/Arg72 or Pro/Pro72 versus Arg/Arg72; OR 2.25, 95% CI 1.21-4.17) or a G:C[&gt;]T:A mutation in TP53 (Pro/Arg72 or Pro/Pro72 versus Arg/Arg72; OR 2.42, 95% CI 0.97-6.04). An interaction between the XPD variant alleles (Asn312 and Gln751) and the TP53 Pro72 allele was observed for TP53 mutations (any TP53 mutation Pint = 0.027, G:C[&gt;]T:A TP53 mutation Pint = 0.041). The statistical interaction observed in our study is consistent with the observed biological interaction for XPD and p53 in nucleotide excision repair and apoptosis. In conclusion, differences in TP53 mutation spectra in lung tumors are associated with several genetic factors and may reflect differences in lung cancer susceptibility and carcinogenesis.


Previously, we demonstrated that connexins (Cxs) showed aberrant localization and expression in most endometrial hyperplasia and carcinoma samples, indicating that during endometrial carcinogenesis, loss of gap junctional intercellular communication (GJIC) may occur at relatively early stages. In the present study, we focused on the correlations between GJIC and the expression of the E-cadherin and its 5' CpG island methylation in endometrial cancer cells and tissues to investigate their roles in the carcinogenesis and tumor progression of endometrial cancer. In this study, three of the 10 cell lines investigated, Ishikawa, RL-952 and KLE, in which both Cxs and E-cadherin mRNA were expressed, exhibited GJIC by scrape-loading/dye transfer. On the other hand, the other seven cell lines, in which either or both Cxs and E-cadherin mRNA were negative or weakly expressed, did not show GJIC. HEC-50, HEC-1B and HEC-108, in which Cxs were positively expressed but E-cadherin was negatively expressed, showed cytoplasmic localization of Cxs by immunohistochemistry. All five lines, which showed the weak expression of E-cadherin, had E-cadherin 5' CpG island methylation. By immunohistochemistry of 56 endometrial carcinomas, 13 of 27 methylated samples showed weak expression of Cx26 and the other 14 showed diffuse localization in cytoplasm. On the other hand, of 29 unmethylated samples, two showed cell-cell localization, 25 weak expression and two diffuse localization. Furthermore, E-cadherin expression was revealed to be drastically down-regulated by E-cadherin antisense oligonucleotides that post-transcriptionally down-regulated E-cadherin expression and in the cell, the localization of Cxs were changed from the cell-cell borders to the cytoplasm, and GJIC also decreased. The results indicated that 5' CpG island methylation, which caused loss of E-cadherin expression, indirectly caused the suppression of GJIC by aberrant localization of Cxs.
Frequent consumption of fresh fruit and vegetables, and polymorphisms in the detoxifying enzyme glutathione S-transferase M1 (GSTM1) and other metabolic genes have been shown to modulate cancer risk at some sites. We have shown recently that DNA adducts, a reliable indicator of genotoxic damage and, possibly, of cancer risk, are modulated by plasma levels of selected micronutrients. Here we further investigate the association between DNA adduct levels and consumption of major food groups and foods, and the estimated dietary intake of nutrients, taking into account the possible modifying effect of metabolic polymorphisms, in a larger sample of 634 healthy adults enrolled in a prospective study in Italy. DNA adducts and five polymorphic metabolic genotypes (GSTM1, GSTT1, NAT2, CYP1A1 and MTHFR) were determined in peripheral leukocytes by using 32P-postlabeling technique and PCR methods. DNA bulky adducts (mean: 7.82 \( \pm \) 0.40/109 nt) were detected in 482/634 samples (76.0%). Overall, DNA adduct levels were significantly and inversely associated with the intake of raw leafy vegetables (\( P = 0.02 \)), non-citrus fruits (\( P = 0.04 \)), potassium (\( P = 0.01 \)) and \( \beta \)-carotene (\( P = 0.05 \)). No association was evident with the five genotypes. Stratification by GSTM1 genotype showed strong inverse associations of DNA adduct levels with increasing consumption of all vegetables combined (\( P = 0.04 \)), leafy vegetables (\( P = 0.004 \)), raw leafy vegetables (\( P = 0.002 \)) and fish (\( P = 0.03 \)) among 307 GSTM1-null subjects; strong inverse associations also emerged with estimated dietary intakes of \( \beta \)-carotene (\( P = 0.004 \)), vitamin E (\( P = 0.004 \)), niacin (\( P = 0.02 \)) and potassium (\( P = 0.01 \)). In contrast, no association emerged among 295 subjects with a GSTM1-wild genotype. Overall, statistically significant interactions in predicting DNA adduct levels were observed between the GSTM1-null genotype and consumption of leafy vegetables (\( P = 0.01 \)), white meat (\( P = 0.04 \)), and intake of vitamin C (\( P = 0.04 \)), vitamin E (\( P = 0.05 \)) and \( \beta \)-carotene (\( P = 0.02 \)). Our results suggest that the role of a diet rich in antioxidants in preventing or reducing DNA adduct formation is restricted to subjects lacking the detoxifying activity of GSTM1 isoenzyme ([\(~\)]50% of the general population).


http://carcin.oupjournals.org/cgi/content/abstract/24/4/739

DNA adducts, a reliable indicator of internal dose exposure to genotoxic agents and, possibly, of cancer risk, have been shown to be modulated by diet, particularly by the consumption of fresh fruit and vegetables, and by the intake of antioxidants (Palli et al., 2000, Int. J. Cancer, 87, 444-451). We have therefore investigated the association between DNA adducts in peripheral leukocytes and plasma levels of selected micronutrients, also taking into account the role of metabolic polymorphisms and smoking history, in a large independent random sample of volunteers enrolled in the prospective study EPIC-Italy ([\(~\)]110 subjects from each of the three main geographical study areas, Northern, Central and Southern Italy). DNA adducts and five polymorphic metabolic genotypes were determined in peripheral leukocytes using the 32P-postlabelling technique and PCR methods. Plasma levels of six carotenoids, retinol and \( \alpha \)- and \( \gamma \)-tocopherol were determined in the same blood sample. Among 331 subjects, 78.3% had detectable levels of DNA adducts (mean 7.46 \( \pm \) 0.48 per 109 nucleotides). Vitamin supplementation was reported by only a few subjects (3.9%). Strong inverse associations emerged between levels of DNA adducts and plasma retinol (\( P = 0.02 \)), \( \alpha \)-tocopherol (\( P = 0.02 \)), and \( \gamma \)-tocopherol (\( P = 0.02 \)).
0.04) and (gamma)-tocopherol (P = 0.03), but not carotenoids (except a borderline inverse association with {beta}-carotene, P = 0.08). An inverse significant association with plasma levels of retinol and (gamma)-tocopherol persisted in the subgroup of non-smokers, whereas a negative association with (alpha)-tocopherol emerged only in smokers. DNA adduct levels did not show any significant variation according to analyzed genotypes. Stratification by GSTM1 genotype, however, showed a significant negative association between DNA adduct levels and plasma levels of (alpha)- (P = 0.02) and (beta)-carotene (P = 0.02) in subjects with the GSTM1 null genotype. Our results confirm that biomarkers of dietary intake of antioxidants significantly modulate DNA adducts and suggest specific inverse associations between DNA adduct levels and antioxidant concentrations among GSTM1 null subjects and smokers.


http://carcin.oupjournals.org/cgi/content/abstract/23/6/1057

Infection with human papillomavirus (HPV) of specific high-risk type triggers a series of events in target cells, which will eventually lead to development of genital neoplasia. The integration of high-risk HPV DNA into the cell genome has been regarded as a crucial event in tumor progression. With respect to different HPV types, the knowledge of HPV integrated loci is still limited. We have now determined the genomic variation and chromosomal location of HPV 33 DNA in the cell line UT-DEC-1, established from a vaginal mild dysplasia lesion. The viral sequence of the cell line was determined, and a variant of the prototype HPV 33 strain was identified, showing nucleotide substitutions resulting in amino acid changes in the E2, L2 and E4 open reading frames. In late passage UT-DEC-1 cells, a deletion of more than half of the 3' part of E1 and major parts of the E2 and E4 genes provided evidence for integration. The flanking sequences of the integration site were completely homologous to published sequences from chromosomal band 5p14, and remained unchanged in all subclones established from late passage cells. There were no chromosomal deletions or gross rearrangements at the integration site, and only a single heterozygotic copy of HPV 33 was detected. The karyotype of late passage cells showed only minor changes compared with early passage cells. During passaging of the cell line, there were progressive changes towards a malignant phenotype, and in parallel to this, the cells carrying episomal HPV 33 of the early passages was completely superseded by cells containing the integrated virus. Thus, our results show that this single copy heterozygote integration of HPV 33 into chromosome band 5p14 appears to be associated with emergence of cells escaping senescence, and with growth advantage compared with cells carrying episomal virus.


http://carcin.oupjournals.org/cgi/content/abstract/23/5/735

Sodium butyrate (NaB), a short-chain fatty acid naturally present in the human colon, is able to induce cell cycle arrest, differentiation and apoptosis in colon cancer cells. In addition to these effects, we investigated the effect of NaB on two angiogenesis-related proteins in a colon carcinoma cell line (HT29): vascular endothelial growth factor (VEGF), the most potent angiogenic factor, and hypoxia-inducible factor (HIF)-1{alpha}, the main transcription activator of the VEGF gene, which are both constitutively expressed at high levels in HT29 also in normoxic conditions. NaB treatment had a different effect on VEGF165 and HIF-1{alpha} expression. In fact, it induced a dose-dependent down regulation of the VEGF165 protein level that was not
paralleled by a concomitant down regulation of the corresponding mRNA, suggesting a post-translational regulation of the factor. Conversely, after 24 h of treatment all the tested NaB concentrations reduced the HIF-1(α) protein level, whereas after a longer time of exposure HIF-1(α) level increased in the presence of a high NaB concentration (2 mM) with a concomitant increase in HIF-1(α) mRNA. These results indicate that NaB, besides regulating other fundamental cellular processes, is able to modulate the expression of two important angiogenesis-related molecules and suggested a further possible clinical application of this short-chain fatty acid as an anti-angiogenic compound in association with conventional chemotherapeutic agents.


http://carcin.oupjournals.org/cgi/content/abstract/24/9/1533

Cytochrome P450 1B1 (CYP1B1) is active in the metabolism of estrogens to reactive catechols and of different procarcinogens. Several studies have investigated the relationship between genetic polymorphisms of CYP1B1 and breast cancer risk, however, with inconsistent results. We investigated such an association in postmenopausal Swedish women, with special emphasis on long-term menopausal hormone users, in a large population-based case-control study. We genotyped 1521 cases and 1498 controls for the CYP1B1 single nucleotide polymorphisms (SNPs) m2, m3 and m4 and reconstructed haplotypes. The frequencies of CYP1B1*1, CYP1B1*2, CYP1B1*3 and CYP1B1*4 alleles among controls were estimated to be 0.087, 0.293, 0.444 and 0.175, respectively. It thus appeared that very few haplotypes contained combinations of SNPs at two or three loci and that single SNP genotype data effectively represented haplotypes. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from logistic regression models. We found no overall association between any CYP1B1 genotype and breast cancer risk. The data indicated, however, that women who had used menopausal hormones for 4 years or longer, and carried the CYP1B1*3/*3 genotype may be at increased risk of breast cancer, OR 2.0 (95% CI 1.1-3.5), compared with long-term users without this genotype. We explored the effect of CYP1B1 genotype on breast cancer risk in subgroups defined by body mass index, family history, smoking and catechol-O-methyl transferase genotype, but found no convincing evidence for interaction. In summary, our results strongly indicate that the studied CYP1B1 gene polymorphisms do not influence breast cancer risk overall but may modify the risk after long-term menopausal hormone use.


http://carcin.oupjournals.org/cgi/content/abstract/24/2/335

There is abundant epidemiological evidence that arsenic is an environmental carcinogen related to human cancers of the skin, lung, liver and urinary bladder, in particular. Dimethylarsinic acid (DMA) has also been reported to act as a carcinogen/or a promoter in rat models. To elucidate molecular mechanisms, we conducted an 18 month carcinogenicity study of DMA in p53 heterozygous (+/-) knockout mice, which are susceptible to early spontaneous development of various types of tumors, and wild-type (+/+) C57BL/6J mice. Totals of 88-90 males, 7-8 weeks of age, were divided into three groups each administered 0, 50 or 200 p.p.m. DMA in their drinking water for 18 months. Mice that were found moribund or died before the end of the study were autopsied to evaluate the tumor induction levels, as well as those killed at the end. Both p53+/+ knockout and wild-type mice demonstrated spontaneous tumor development, but lesions were more prevalent in the knockout case. Carcinogenic effect of DMA was evident by significant early
induction of tumors in both treated p53+/− knockout and wild-type mice, significant increase of the tumor multiplicity in 200 p.p.m.-treated p53+/− knockout mice, and by significant increase in the incidence and multiplicity of tumors (malignant lymphomas) in the treated wild-type mice. By the end of 80 weeks, tumor induction, particularly malignant lymphomas and sarcomas, were similar in treated and control p53+/− knockout mice. No evidence for organ-tumor specificity of DMA was obtained. Molecular analysis using PCR-SSCP techniques revealed no p53 mutations in lymphomas from either p53+/− knockout or wild-type mice. In conclusion, DMA primarily exerted its carcinogenic effect on spontaneous development of tumors with both of the animal genotypes investigated here.


http://carcin.oupjournals.org/cgi/content/abstract/24/9/1455

Genetic susceptibility to breast cancer is influenced by high- and low-penetrance genes. The low-penetrance genes contributing to increased and decreased risk likely exist at appreciable frequencies in the human population. To identify high-frequency, low-penetrance modifier genes, we are using a rat genetic model. Eight quantitative trait loci, named mammary carcinoma susceptibility (Mcs) loci, have been genetically identified in two rat strains, Wistar-Kyoto (WKy) and Copenhagen. These strains are resistant to developing mammary cancer compared with susceptible Wistar-Furth (WF) female rats. Here we provide physical evidence of the existence of Mcs5 in the resistant WKy rat and further narrow the candidate region defining the QTL. Two congenic rat lines (C and D) containing large segments of the WKy Mcs5 QTL on chromosome 5 were generated on a WF background. The minimal WKy interval from markers D5Wox7 and D5Uwm37 (line C) conferred resistance to developing 7,12-dimethylbenz-aanthracene (DMBA)-induced mammary carcinomas. Line C females that were homozygous for the WKy allele at this interval averaged 1.1{+/-}0.3 carcinomas/rat compared with 6.9{+/-}0.4 average carcinomas/rat for WF control females (P<0.01). Line D females containing the minimal WKy interval from D5Rat26 to D5Uwm42, were as susceptible to developing mammary carcinomas as WF controls (5.7{+/-}0.6 versus 6.9{+/-}0.4, respectively). The WKy region in common to these lines from D5Rat26 to D5Uwm37 is thus not expected to contain Mcs5-associated genes. Based on results presented here, the Mcs5 locus has been physically located within a congenic interval on rat chromosome 5 between markers D5Uwm8 and D5Rat26.


http://carcin.oupjournals.org/cgi/content/abstract/26/4/855

p33ING1b is a candidate tumor suppressor gene and a nuclear protein. We investigated whether genetic and epigenetic mechanisms affect p33ING1b expression in ovarian cancer thus contributing toward its pathogenesis. A total of 111 ovarian cancers collected from Beijing and Hong Kong were used for this study. Weak or negative p33ING1b protein expression was demonstrated by immunohistochemistry on tissue microarray in 28/111 cases. Real-time quantitative RT-PCR also showed overall significant reduction of p33ING1b mRNA expression (P = 0.0137), with 53.1% (17/32) cases showing 2- to 5-fold reduction and absence of expression. The reduction of mRNA expression in cancer correlated with decreased p33ING1b protein expression (P < 0.0001). While no p33ING1b mutation was found, allelic loss at the p33ING1b locus was demonstrated in 25% (8/32) cases. The allelic loss profiles also showed statistical significant correlation with reduction of p33ING1b protein and mRNA expression (P = 0.031 and 0.030). Promoter methylation as assessed by methylation specific PCR was found in 23.9%
Bisulfite sequencing results confirmed the p33ING1b promoter methylation status of these methylation positive cases. Statistical significant correlation between methylation and mRNA expression (P = 0.006) was demonstrated. Treatment with demethylating drug, 5'-aza-2'-deoxycytidine, resulted in dosage-dependent elevated mRNA expression of p33ING1b in ovarian cancer cell lines. This is the first study reporting epigenetic mechanism regulating the p33ING1b expression. Our findings support that genetic and epigenetic alteration of p33ING1b are likely to contribute towards the pathogenesis of ovarian cancers.


http://carcin.oupjournals.org/cgi/content/abstract/25/12/2311

Oxidized DNA base lesions, such as thymine glycol (Tg) and 8-hydroxyguanine, are often toxic and mutagenic and have been implicated in carcinogenesis. To clarify whether NEIL1 protein, which exhibits excision repair activity towards such base lesions, is involved in gastric carcinogenesis, we examined 71 primary gastric cancers from Japanese patients and four gastric cancer cell lines for mutations and genetic polymorphisms of the NEIL1 gene. We also examined 20 blood samples from Chinese patients for NEIL1 genetic polymorphisms. Three mutations (c.82_84delGAG:p.Glu28del, c.936G > A and c.1000A > G:p.Arg334Gly) and two genetic polymorphisms were identified. When the excision repair activity towards double-stranded oligonucleotide containing a Tg:A base pair was compared among six types of recombinant NEIL1 proteins, p.Glu28del-type NEIL1, found in a primary case, was found to exhibit an extremely low activity level. Moreover, c.936G > A, located in the last nucleotide of exon 10 and detected in the KATO-III cell line, was shown to be associated with a splicing abnormality using an in vivo splicing assay. An immunofluorescence analysis showed that the wild-type NEIL1 protein, but not the truncated protein encoded by the abnormal transcript arising from the c.936G > A mutation, was localized in the nucleus, suggesting that the truncated protein is unlikely to be capable of repairing nuclear DNA. An expression analysis revealed that NEIL1 mRNA expression was reduced in six of 13 (46%) primary gastric cancer specimens that were examined. These results suggest that low NEIL1 activities arising from mutations and reduced expression may be involved in the pathogenesis in a subset of gastric cancers.


http://carcin.oupjournals.org/cgi/content/abstract/23/1/61

Several epidemiological studies have suggested a modulatory effect of dietary folate intake on the risk of colorectal cancer. The molecular basis for this inverse association is not clearly understood, but may involve alterations in DNA methylation. In this study, we examined the levels of methylation intermediates [S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH)] and of global DNA methylation in the pre-neoplastic small intestine of Min (multiple intestinal neoplasia) mice. We also studied the effect of folate/choline deficiency on these parameters and on tumor multiplicity in this animal model. In folate-adequate Min mice, we identified positive linear correlations between SAM or SAH and tumor numbers (R2 = 0.38, P < 0.005; R2 = 0.26, P = 0.025, respectively). A positive correlation between global DNA hypomethylation and tumor multiplicity was also observed (R2 = 0.29, P = 0.014). These three biochemical determinants (SAM, SAH and DNA hypomethylation) may, therefore, serve as early markers of cell transformation. Folate/choline deficiency, however, did not produce a consistent effect on tumor numbers in three separate experiments. As an increase in tumor numbers was observed only in

http://carcin.oupjournals.org/cgi/content/abstract/25/9/1629

The isothiocyanate, sulforaphane and the flavonoid, apigenin modulate gene expression including phase II detoxifying enzymes, such as glutathione S-transferases (GST) and UDP-glucuronosyltransferases (UGT). Using undifferentiated CaCo-2 cells, we have examined the interactions between sulforaphane and apigenin in the regulation of UGT and GST expression. We show that apigenin induces UGT1A1 transcription (4-fold) but not GSTA1, and that sulforaphane induces both UGT1A1 (3.7-fold) and GSTA1 (2.8-fold) transcription in both dose- and time-dependent manners. The combination of sulforaphane and apigenin resulted in a synergistic induction of UGT1A1 mRNA up to 12-fold, although this interaction was not seen for GSTA1. Nuclear factor kappa B (NF-(kappa)B) mRNA was induced by apigenin and sulforaphane (2.5- and 2-fold, respectively). NF-(kappa)B translocation inhibitor SN50 and phosphatidylinositol 3-kinase (PI3) inhibitor LY294002 decreased the induction of GSTA1 by sulforaphane almost to baseline level. However, the MEK inhibitor PD98059 enhanced significantly the induction of GSTA1 by sulforaphane. This suggests that NF-(kappa)B and PI3-kinase signaling pathways play a role in GSTA1 gene expression. Conversely, the induction of UGT1A1 transcription by sulforaphane was totally abolished by PD98059, although PD98059 slightly enhanced (20%) the induction of UGT1A1 by apigenin implying that the induction of UGT1A1 by sulforaphane is mediated by MAPK/extracellular signal-regulated kinase kinase, whereas UGT1A1 induction by apigenin may be associated with NF-(kappa)B translocation since the NF-(kappa)B translocation inhibitor, SN50 enhanced the induction of UGT by apigenin. The results show that UGT1A1 and GSTA1 are regulated by sulforaphane through different signal transduction pathways and the differences in the mechanisms of modulation of UGT1A1 transcription by sulforaphane and apigenin resulted in a synergistic effect between these two compounds in the induction of UGT1A1.


http://carcin.oupjournals.org/cgi/content/abstract/24/1/39

Although a number of studies have suggested that diets with low intake of folate, an important methyl donor, are associated with increased risks of colon cancer and its precursor the adenomatous polyp, the underlying mechanisms are poorly understood. Dysregulation and instability of DNA methylation and alterations in the levels of the predominant DNA methylating enzyme, DNA (cytosine-5)-methyltransferase 1 (Dnmt1), have also been linked to tumorigenesis. We have used a combination of genetic and dietary manipulation to assess the effects of reduced Dnmt1 expression with and without folate deficiency on tumor induction in the ApcMin mouse. ApcMin mice with a reduction in Dnmt1 expression (ApcMin/+Dnmt1C+/+) had significantly lower tumor numbers than ApcMin mice with normal Dnmt1 (ApcMin/+Dnmt1+/+). Dietary folate deficiency from weaning to 13 weeks of age did not affect tumor number or size in ApcMin/+Dnmt1+/+ mice. However, in ApcMin/+Dnmt1C+/+ mice with high baseline tumor numbers (41 +/- 4), folate deficiency was associated with a decreased absolute number of tumors (27 +/- 3), but a higher proportion of larger tumors as compared with mice on the control diet. In the repeat experiment, ApcMin/+Dnmt1C+/+ mice had low baseline tumor numbers (20 +/- 2) and folate deficiency did not affect tumor number (23 +/- 4) or size as compared with the
same mice on the control diet. These results suggest that, in the presence of Dnmt1 deficiency, the effects of folate deficiency on tumor number and size may depend on the stage of adenoma development when folate deficiency is initiated. We also show that folate deficiency with or without reductions in Dnmt1 did not affect overall genomic DNA methylation or the methylation levels of two candidate genes, E-cadherin or p53, in normal or neoplastic intestinal tissue. In conclusion, genetic deficiency in Dnmt1 with or without folate deficiency decreases tumor number in the ApcMin mouse model, but this effect may not be mediated by changes in SAM or SAH levels, nor by alterations in global methylation in the pre-neoplastic intestinal tissue.


To investigate a possible link between bile acids and the pathogenesis of pancreatic cancer, we determined whether conjugated or unconjugated bile acids induced cyclooxygenase-2 (COX-2) in two human pancreatic cancer cell lines, BxPC-3 and SU 86.86. Bile acids are known promoters of gastric and colon cancer. We demonstrated previously that COX-2, an enzyme that catalyzes the synthesis of prostaglandins, is over-expressed in human pancreatic adenocarcinoma. Both human pancreatic cell lines were treated with conjugated and unconjugated bile acids. COX-2 mRNA and protein were determined. In addition, prostaglandin E2 (PGE2) synthesis was measured. Treatment with conjugated or unconjugated bile acids for 3 h up-regulated COX-2 mRNA. Chenodeoxycholate (CD) or deoxycholate at concentrations ranging from 12.5 to 100 \{micro\}M caused a dose-dependent induction of COX-2 protein with a maximal effect at 100 \{micro\}M. Induction of COX-2 protein by CD and deoxycholate was detected after treatment for 6 h with maximal induction at 12 h. Taurochenodeoxycholate, a conjugated bile acid, also caused dose-dependent induction of COX-2 but higher concentrations of bile acid (200-1200 \{micro\}M) were required. Levels of cyclooxygenase-1 were unaffected by bile acid treatment. Unconjugated and conjugated bile acids caused 7- and 4-fold increases in PGE2 production, respectively. Taken together, these findings suggest a possible role for bile acids in the pathogenesis of pancreatic cancer.


Genetic instability is a prominent feature in multiple myeloma and progression of this disease from monoclonal gammopathy of uncertain significance (MGUS) and smouldering myeloma (SMM) is associated with increasing molecular and chromosomal abnormalities. The DNA mismatch repair (MMR) pathway is a post-replication DNA repair system that maintains genetic stability by repairing mismatched bases and insertion/deletion loops mistakenly incorporated during DNA replication. Deficiencies in proteins pivotal to this pathway result in a higher mutation rate, particularly at regions of microsatellite DNA. We have investigated the proficiency of the MMR pathway in clinical samples and myeloma cell lines. Microsatellite analysis showed instability at one or more of nine loci examined in 15 from 92 patients: 7.7% of MGUS/SMM, 20.7% of MM/plasma cell leukaemia (PCL) and 12.5% of relapsed MM/PCL. An in vitro heteroduplex G/T repair assay found reduced repair in two cell lines, JIM1 and JIM3, and in two of four PCL cases and was associated with aberrant expression of at least one mismatch repair protein. Thus we show that MMR defects are found in plasma cell dyscrasias and the increased frequency during more active stages of the disease suggests a contributory role in disease progression.
Estrogen is involved in breast carcinogenesis. Hypotheses have been raised that its effect is modified by enzymes such as catechol-O-methyltransferase (COMT) that deactivate potentially genotoxic estrogen metabolites. We have investigated the association between the functional genetic Val108/158Met polymorphism in COMT and breast cancer risk in a large population-based case-control study performed in the genetically homogeneous Swedish population. We determined COMT genotype in 1534 women with invasive breast cancer and in 1504 control women and calculated odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models. There was no overall association between COMT genotype and breast cancer risk. However, the L allele was associated with an increased risk for lobular breast cancer, with OR 2.0 (95% CI 1.2-3.5) for HL and 1.7 (95% CI 0.9-3.0) for LL. In exploratory subset analyses, we found no statistically significant interaction, but some indication of a positive association between HL and LL genotypes and breast cancer among women with diabetes mellitus and a negative association among nulliparous women. Based on our findings, COMT activity alone does not seem to play a major role in breast carcinogenesis, but may be of importance in certain histotypes or in conjunction with other exposures.

Arsenic is a well-documented human carcinogen, and contamination with this heavy metal is of global concern, presenting a major issue in environmental health. However, the mechanism by which arsenic induces cancer is unknown, in large part due to the lack of an appropriate animal model. In the present set of experiments, we focused on dimethylarsinic acid (DMA), a major metabolite of arsenic in most mammals including humans. We provide, for the first time, the full data, including detailed pathology, of the carcinogenicity of DMA in male F344 rats in a 2-year bioassay, along with the first assessment of the genetic alteration patterns in the induced rat urinary bladder tumors. Additionally, to test the hypothesis that reactive oxygen species (ROS) may play a role in DMA carcinogenesis, 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation in urinary bladder was examined. In experiment 1, a total of 144 male F344 rats at 10 weeks of age were randomly divided into four groups that received DMA at concentrations of 0, 12.5, 50, and 200 p.p.m. in the drinking water, respectively, for 104 weeks. From weeks 97-104, urinary bladder tumors were observed in 8 of 31 and 12 of 31 rats in groups treated with 50 and 200 p.p.m. DMA, respectively, and the preneoplastic lesion, papillary or nodular hyperplasias (PN hyperplasia), was noted in 12 and 14 rats, respectively. DMA treatment did not cause tumors in other organs and no urinary bladder tumors or preneoplastic lesions were evident in the 0 and 12.5 p.p.m.-treated groups. Urinary levels of arsenicals increased significantly in a dose-responsive manner except for arsenobetaine (AsBe). DMA and trimethylarsine oxide (TMAO) were the major compounds detected in the urine, with small amounts of monomethylarsonic acid (MMA) and tetramethylarsonium (TeMa) also detected. Significantly increased 5-bromo-2'-deoxyuridine (BrdU) labeling indices were observed in the morphologically normal epithelium of the groups treated with 50 and 200 p.p.m. DMA. Mutation analysis showed that DMA-induced rat urinary bladder tumors had a low rate of H-ras mutations (2 of 20, 10%). No alterations of the p53, K-ras or (beta)-catenin genes were detected. Only one TCC (6%) demonstrated nuclear accumulation of p53 protein by immunohistochemistry. In 16 of 18 (89%) of the TTCs and 3 of 4 (75%) of the papillomas, decreased p27kip1 expression could be demonstrated. Cyclin D1 overexpression
was observed in 26 of 47 (55%) PN hyperplasias, 3 of 4 (75%) papillomas, and 10 of 18 (56%) TCCs. As a molecular marker of oxidative stress, increased COX-2 expression was noted in 17 of 18 (94%) TCCs, 4 of 4 (100%) papillomas, and 39 of 47 (83%) PN hyperplasias. In experiment 2, 8-OHdG formation in urinary bladder was significantly increased after treatment with 200 p.p.m. DMA in the drinking water for 2 weeks compared with the controls. The studies demonstrated DMA to be a carcinogen for the rat urinary bladder and suggested that DMA exposure may be relevant to the carcinogenic risk of inorganic arsenic in humans. Diverse genetic alterations observed in DMA-induced urinary bladder tumors imply that multiple genes are involved in stages of DMA-induced tumor development. Furthermore, generation of ROS is likely to play an important role in the early stages of DMA carcinogenesis.


http://carcin.oupjournals.org/cgi/content/abstract/26/2/395

Glutathione S-transferases detoxify polycyclic aromatic hydrocarbons found in tobacco smoke by glutathione conjugation. Polymorphisms within the GSTM1, GSTT1 and GSTP1 genes, coding for enzymes with deficient or reduced activity, have been studied as potential modifiers of lung cancer risk. It is hypothesized that risk associated with potential susceptibility gene polymorphisms might be most evident at low levels of exposure. Never smokers developing lung cancer represent a highly susceptible subset of the population, exposed to tobacco carcinogens only through environmental tobacco smoke. This population-based case-control study examines the association between GSTM1, GSTT1 and GSTP1 genotypes and lung cancer in one of the largest samples of never smokers to date. Cases (n = 166) were identified through the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and age- and race-matched population-based controls (n = 181) were identified using random digit dialing. Overall, there was no significant association between single or combinations of genotypes at GSTM1, GSTT1 or GSTP1 and lung cancer risk after adjustment for age, race, sex and household ETS exposure in years. However, in never smokers exposed to 20 or more years of household ETS, carrying the GSTM1 null genotype was associated with a 2.3-fold increase in risk [95% confidence interval (CI) 1.05-5.13]. Individuals in this high ETS exposure category carrying the GSTM1 null and the GSTP1 Val allele were at over 4-fold increased risk of developing lung cancer (OR = 4.56, 95% CI: 1.21-17.21). These findings suggest that in the presence of ETS, the GSTM1 genotype both alone and in combination with the GSTP1 genotype alters the risk of developing lung cancer among never smokers.


http://carcin.oupjournals.org/cgi/content/abstract/25/10/1935

Multiple enzymes with overlapping functions and shared substrates in the glutathione (GSH) metabolic pathway have been associated with host susceptibility to tobacco smoke carcinogens and in lung cancer etiology. However, few studies have investigated the differing and interacting roles of GSH pathway enzymes with tobacco smoke exposure on lung cancer risk in young (<50 years of age) and old (>80 years of age) populations. Between 1997 and 2001, 237 primary lung cancer patients (170 young, 67 old) and 234 controls (165 young, 69 old) were enrolled at the Mayo Clinic. Using PCR amplification of genomic DNA, polymorphic markers for {gamma}GCS, GPX1, GSTP1 (I105V and A114V), GSTM1 and GSTT1 were genotyped. Recursive partitioning and logistic regression models were used to build binary classification trees and to estimate odds
ratios (OR) and 95% confidence intervals for each splitting factor. For the young age group, cigarette smoking had the greatest association with lung cancer (OR = 3.3). For never smokers, the dividing factors of recursive partitioning were GSTT1 (OR = 1.7), GPX1 (OR = 0.6) and GSTM1 (OR = 4.3). For the old age group, smoking had the greatest association with lung cancer (OR = 3.6). For smokers, the dividing factors were GPX1 (OR = 3.3) and GSTP1 (105V) (OR = 4.1). Results from logistic regression analyses supported the results from RPART models. GSH pathway genes are associated with lung cancer development in young and old populations through differing interactions with cigarette smoking and family history. Carefully evaluating multiple levels of gene-environment and gene-gene interactions is critical in assessing lung cancer risk.

**Cardiovascular Pathology**  (1)


http://www.sciencedirect.com/science/article/B6T13-3YVMYPJ-14/2/a3401c4d91caf866a77835241ddd6581

Viral infection of the myocardium is implicated in the pathogenesis of myocarditis and dilated cardiomyopathy (DCM). Enteroviruses have been considered the most common viral etiologic agents, based on peripheral culture and serologic methods. Recently, polymerase chain reaction (PCR) has been shown to be useful in the detection of viral genomes from various infected organs and body fluids. In this study, myocardial samples from autopsy specimens (formalin fixed and fresh frozen) were examined for entervoiral and DNA viral (adenovirus, herpes simplex virus [HSV], and cytomegalovirus (CMV)) genome by PCR. The specimens studied were from 58 patients with myocarditis, 28 patients with DCM and endocardial fibroelastosis [EFE], and 22 controls. Viral genome was detectable in 34 of the 58 (59%) autopsy-proven myocarditis samples (18 adenovirus, 12 enterovirus, 2 CMV, 2 HSV) and 6 of the 28 samples from patients with DCM and EFE (6 adenovirus). We conclude that PCR is effective in the rapid amplification of virus from frozen and formalin-fixed myocardial samples and that adenovirus is an important etiologic agent in viral myocarditis as well as DCM with EFE.

**Cardiovascular Research**  (13)


http://www.sciencedirect.com/science/article/B6T14-3TCFMDY-N/2/855e6599f570a441be0195db7d10ef44
Objective: Methylation of cytosine in CG dinucleotides within regulatory elements is believed to silence gene expression. These dinucleotides occur in certain important regulatory elements in the promoter region of the human [beta]-myosin heavy chain ([beta]-MHC) gene. We therefore investigated whether methylation of these elements correlates with [beta]-MHC gene transcription in human 'expressing' (right atrial) and 'non-expressing' (peripheral blood leucocytes) cells.

Methods: We employed 2 techniques to assess promoter methylation: (i) analysis of the susceptibility to digestion of a particular CCGG restriction site in the promoter region when genomic DNA is cleaved with the restriction endonucleases MspI (methylation-insensitive) and HpaII (methylation-sensitive), and (ii) the bisulphite-PCR method to examine in detail the methylation patterns of 3 important regulatory elements that contain CG dinucleotides. [beta]-MHC mRNA expression in right atrium and leucocytes was assessed using reverse-transcription-PCR with specific primers that do not detect [alpha]-MHC cDNA. Results: The digestion pattern observed with MspI or HpaII indicated that the CCGG site was almost completely methylated in leucocytes, but relatively unmethylated in atrial myocardium from the same patients. When methylation was examined with the bisulphite-PCR method we found a reciprocal relationship between the level of [beta]-MHC mRNA expression in leucocytes and atrial myocardium and the degree of methylation of CG dinucleotides in the 5' regulatory elements of the gene. Conclusions: Tissue-specific methylation of the human [beta]-MHC gene promoter may play a role in determining the pattern of expression of this gene. Furthermore, alteration of the level of methylation may underlie the changes in transcription of this gene that occur, for example, when atrial or ventricular myocardium hypertrophies.


http://www.sciencedirect.com/science/article/B6T14-448BCB1-H/2/c65dddc22d5633849bb199b0fab52488

Objective: Increasing evidence suggests that vascular calcification is a regulated process. We studied the vascular expression pattern of a key factor in mineralization and a counteracting, protective factor. Based on the phenotype of null mice, Core binding factor [alpha]-1 (Cbfa-1) plays a pivotal role in bone formation, whereas Matrix Gla Protein (MGP) is a potent inhibitor of vascular calcification. Methods: We investigated the expression of MGP and Cbfa-1 in cultured, human monocyct cells, endothelial cells and smooth muscle cells (SMC), as well as in normal and atherosclerotic vessel specimens. Results: In cultured cells MGP is expressed in endothelial cells and SMC, whereas Cbfa-1 mRNA is predominantly present in macrophages and to a lesser extent in SMC. In the normal vessel wall MGP expression is high at the luminal side and declines toward the center of the media, whereas Cbfa-1 is absent. Moderate, diffuse calcification of the aorta media was observed only in those regions where MGP is low or absent. In atherosclerotic lesions MGP is expressed in endothelial cells and SMC that form fibrous caps, but is never present in macrophages. Cbfa-1 mRNA is predominantly present in macrophages and to a lesser extent in SMC. In the normal vessel wall MGP expression is high at the luminal side and declines toward the center of the media, whereas Cbfa-1 is absent. Moderate, diffuse calcification of the aorta media was observed only in those regions where MGP is low or absent. In atherosclerotic lesions MGP is expressed in endothelial cells and SMC that form fibrous caps, but is never present in macrophages. Cbfa-1 mRNAs in regions without MGP, it is associated with calcified areas and Cbfa-1 may be considered a marker for osteoprogenitor-like cells in the vessel wall. Conclusions: Our observations on MGP expression confirm and extend published data and are consistent with a protective function of MGP. Cbfa-1 expression is absent in normal medial SMC and co-localizes with neointimal macrophages and focal calcifications.


http://www.sciencedirect.com/science/article/B6T14-3R37WSS-S/2/8bffb5bb805915b3bb94aabe159e77f2c
Objective: While natriuretic peptides can inhibit growth of vascular smooth muscle cells (VSMC), controversy exists as to whether this effect is mediated via the guanylate cyclase-coupled receptors, NPR-A and NPR-B, or the clearance receptor, NPR-C. The original aim of this study was to examine the mechanism by which the NPR-C receptor regulates growth. Methods: Rat VSMC were characterized with regard to natriuretic peptide receptor expression by RT/PCR and radioligand binding studies. The effect on growth following addition of the peptides and the ligands for NPR-C was measured by [3H]thymidine incorporation. Cyclic guanosine monophosphate (cGMP) levels were determined by radioimmunoassay and mitogen activating protein kinase activity was based on the phosphorylation of myelin basic protein. Results: In rat VSMC, passages 4-12, both atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) dose-dependently inhibited serum and PDGF-induced VSMC growth. In contrast, NPR-C specific ligands alone had no effect on cell growth but enhanced growth inhibition when co-administered with ANP and CNP. ANP and CNP also decreased PDGF-BB-stimulated MAP kinase activity. Once again, NPR-C specific ligands alone had no effect but enhanced the effects of ANP. Furthermore, a cGMP specific phosphodiesterase inhibitor dose-dependently inhibited VSMC growth and markedly enhanced natriuretic-peptide-induced inhibition at low peptide concentrations. To examine a potential mechanism for the controversy concerning the NPR-C, we investigated the autocrine expression of ANP and CNP by VSMC and found that mRNA encoding both peptides could be detected by RT/PCR. Conclusion: Our findings indicate that the guanylyl-cyclase-linked receptors mediate the antiproliferative actions of the natriuretic peptides on vascular smooth muscle cell growth. Moreover, we hypothesize that the apparent inhibition of growth by NPR-C specific ligands reported by others may be due to stabilization of natriuretic peptides produced by the cultured VSMC and subsequent action of these peptides at guanylyl-cyclase-linked receptors.


http://www.sciencedirect.com/science/article/B6T14-3YGV5CF-13/2/65d3595d32fcee1770afc9795a10868c

Objective: The function of angiotensin converting enzyme (ACE) at cell sites of high collagen turnover, such as heart valves, is uncertain. The aim of this study was to assess ACE and kininase-II-like activities and collagen turnover in cultured valvular interstitial cells of the adult rat heart. Methods: The valvular interstitial cell phenotype was determined by immunolabelling (rhodamine phalloidin, desmin, and Griffonia simplicifolia lectin), and the presence of ACE mRNA and protein was confirmed by reverse transcriptase-polymerase chain reaction analysis, ACE monoclonal antibody and in vitro autoradiography, respectively. ACE and kininase-II-like activities in valvular interstitial cells were analysed by high performance liquid chromatography. Angiotensin II (AT1) and bradykinin receptors in valvular interstitial cell membranes were examined by western immunoblotting and binding assay. Type I collagen and collagenase in valvular interstitial cell culture media were determined by and zymography, respectively. Type I collagen mRNA expression in cultured valvular interstitial cells was determined by northern blot analysis and in situ hybridisation. Results: In intact valvular interstitial cells or their cell membrane we found: (1) actin microfilaments, but not desmin or lectin labelling; (2) ACE mRNA expression and binding activity; (3) conversion of angiotensin I to angiotensin II, which was completely inhibited by 50 [mu]M lisinopril, while kininase-II-like activity exceeded ACE activity and was not inhibited by lisinopril; (4) AT1 and bradykinin receptors in valvar interstitial cell membrane preparations; (5) type I collagen mRNA expression and collagenase activity; and (6) angiotensin II induced increase in type I collagen synthesis and mRNA expression. Conclusions: Cultured valvular interstitial cells represent a non-endothelial, non-smooth-muscle cell type that expresses mRNA for ACE and type I collagen. ACE and kininase-II-like activities in valvar interstitial cells may be involved in the regulation of peptides that influence collagen turnover. Angiotensin II stimulates type I collagen synthesis and mRNA expression in these cells.

http://www.sciencedirect.com/science/article/B6T14-3X1182G-10/2/9f9280e5ddeb4a7c6d624db6ef4e8acb

Objective: Congestive heart failure (CHF) is accompanied by enhanced peripheral sympathetic nerve activity, increased vascular resistance and impaired peripheral blood flow. Besides noradrenaline and neuropeptide Y, the sympathetic nervous system also releases ATP, which has contractile effects mediated by different subtypes of P2-receptors on the vascular smooth muscle cells. The present study was designed to examine postsynaptic changes of the contractile responses to ATP and other extracellular nucleotides in CHF. Methods: CHF was induced by left coronary artery ligation resulting in a reproducible myocardial infarction in Sprague-Dawley rats. Contractile responses were examined in cylindrical segments of aorta and the mesenteric artery after endothelium removal. To determine if an altered response was regulated on the transcriptional level, competitive reverse transcription polymerase chain reaction (RT-PCR) was used to estimate the amount of P2X1-receptor mRNA. Results: ATP, which is both a P2X1- and a P2Y-receptor agonist, induced a weaker contraction in the mesenteric artery from CHF as compared to sham operated rats. A decrease in both potency and maximum contraction was shown for the selective P2X1-receptor agonist, [alpha][beta]-MeATP, in the mesenteric artery (pEC50=6.04 vs. 5.76, Cmax=57% vs. 33%, sham vs. CHF operated rats), but not in the aorta. Competitive RT-PCR also revealed decreased P2X1-receptor mRNA levels in CHF operated rats in the mesenteric artery (9106.103 vs. 714.103 molecules/µg, sham vs. CHF operated rats), while it remained unaltered in the aorta. To study the P2Y-receptor induced contractile effects, the P2X1-receptors were first desensitised with [alpha][beta]-MeATP (10⁻⁵ M for 8 min). After P2X1-receptors desensitisation, UTP and UDP induced strong contractions in both the mesenteric artery and in the aorta, while ATP and ADP were much less effective. These contractions were not altered by CHF, indicating that vascular contraction mediated by P2Y-receptors are unaffected by CHF. Conclusion: CHF induces downregulation of P2X1-receptor stimulated contraction in the mesenteric artery depending on decreased mRNA synthesis for the receptor, while the P2Y-receptor activity remains unchanged. Downregulation of P2X1-receptors appears to be specific for peripheral resistance arteries. This may represent a compensatory response to enhanced peripheral sympathetic nerve activity and increased vascular resistance in CHF.


http://www.sciencedirect.com/science/article/B6T14-3VXNC13-G/2/72dd0f681086d8ad6cf18cdf209e3b12

Objective: Inflammatory cells invade the fibrotic myocardium of spontaneously hypertensive rats at the same sites as where fibroblasts are produced. The role of these inflammatory cells in myocardial fibrogenesis was studied in the present work. Methods: The production and distribution of proteins that may be implicated in inflammation was examined by immunohistochemistry of sections of left ventricles from 1-month and 4-month renovascular hypertensive and age-matched control rats using antibodies against ICAM-1, LFA-1, TGF[beta]1, PDGF-A, T and H kininogens, IgG, IgM, C3, and C5b-9. Infiltrating inflammatory cells were phenotyped by immunohistochemistry. The TGF[beta]1 and PDGF-A mRNA levels were checked by RT-PCR. Results: Infiltrating cells were mainly T helper lymphocytes and macrophages, and there were more inflammatory cells in hypertensive rats than in control rats, localized especially around coronary arteries and in microscars. There were more ICAM-1 and LFA-1 in the ventricles.
of hypertensive than in control rats at 1 month, but the ICAM-1 expressions in hypertensive and control rats were similar at 4 months. TGF[beta]1 and PDGF-A mRNA steady states increased in 4-month hypertensive rats, but there was no labeling for TGF[beta] or PDGF by immunohistochemistry. There was only faint labeling for T and H kininogens, and it was not increased in hypertensive rats. There were deposits of IgM and C5b-9 only in hypertensive rats. Conclusion: Thus, inflammatory cells infiltrate the cardiac tissue of renovascular hypertensive rats as in the case of spontaneously hypertensive rats and these cells may use the ICAM-1/LFA-1 system to infiltrate, but neither TGF[beta] and PDGF-A, nor the kininogen system seem to be associated with cardiac fibrogenesis. Otherwise, the complement system could act as arteriosclerotic and/or leukocyte mobilizing factors.


Objective: The aim of this study was to determine the role of AMP-activated protein kinase (AMPK) and its link to protein kinase C (PKC) in the late phase of cardioprotection afforded by ischemic preconditioning (PC) against myocardial stunning. Methods and results: Rabbits were instrumented with a balloon occluder around a coronary artery and with a Doppler sensor to monitor the thickening fraction (TF). Conscious rabbits underwent five cycles of 5-min ischemia/5-min reperfusion (I/R) on 2 consecutive days (days 1 and 2). Reduction of TF after I/R was significantly less and recovery of TF was faster on day 2, indicating a late PC effect. PC provoked translocation of PKC-ɛ from the cytosol to the membrane and significantly increased AMPK activity by 100% immediately after PC. The mRNA level of GLUT4, a glucose transporter, was elevated by 150% at 3 h after PC, and the total protein level of GLUT4 was increased by 107% at 24 h after PC. The level of sarcolemmal GLUT4 protein after I/R on day 2 was 41% higher than its level after I/R on day 1. AMPK activation and up-regulation of GLUT4 by PC were abrogated by pre-treatment with PKC inhibitors. Conclusion: PC activated AMPK and up-regulated GLUT4 expression in a PKC-dependent manner. This GLUT4 up-regulation at 24 h after PC may contribute to attenuation of myocardial stunning.


http://www.sciencedirect.com/science/article/B6T14-3T11M0P-H/2/7d66aea2744aa259776a5181b36322a6

Objective: Analysis of T-cell receptor (TCR) [beta]-chain gene expression in atherosclerotic lesions of human aorta. Methods: TCR diversity was studied using non-radioactive polymerase chain reaction for quantitative assessment of TCRBV gene transcripts, together with size and sequence analysis of the [beta]-chain third complementarity-determining region (CDR3). Samples represent a wide range of atheromatous histology, allowing evaluation of the T-cell repertoire at different stages of disease. Results: Diverse TCRBV family usage was observed in the majority of the samples, as the 25 different TCRBV products were detected at levels exceeding background. The data also showed that TCRBV transcripts expressed in the diseased aorta tissue displayed considerable size heterogeneity and no repetition of CDR3 nucleotide motifs. Conclusions: The early presence of T-lymphocytes in the atheromatous blood vessel has been interpreted as an indication of specific immunological reactions operating during the course of the atherosclerotic process. Although a T-cell infiltrate characterized by limited usage of TCRAV genes cannot be
excluded, the unrestricted usage of TCRBV genes argues against a local T-cell clonal expansion in atherogenesis.


http://www.sciencedirect.com/science/article/B6T14-460MB58-7/2/439344a52a4f1c2ad6ac6e7e0dd6876c

Objective: Two major isoforms of smoothelin have been reported, a 59-kDa smoothelin-A in visceral smooth muscle cells and a 110-kDa smoothelin-B in vascular smooth muscle cells. The present study was undertaken to investigate the expression of these smoothelin isoforms in different smooth muscle tissues and to determine how they are generated. Methods: Western blotting with a new, well-defined, smoothelin antibody was used to confirm the existence of two major smoothelin isoforms. Northern blotting, RT-PCR, primer extension and 5'RACE were applied to analyse the expression of these isoforms in human and mouse. Promoter reporter assays were carried out to establish the existence of a dual promoter system governing the expression pattern of the gene. Results: Antibody C6G confirmed the existence of two smoothelin proteins. Northern blotting showed that in vascular tissues a larger smoothelin transcript is generated than in visceral tissue. The cDNA of this larger smoothelin-B was cloned. Computer analysis of the open reading frame suggests an [alpha]-helical structure of 130 amino acids at the amino terminus of smoothelin-B. The smoothelin gene was cloned and sequenced. It comprises about 25 kb and contains 21 exons. The translational start of smoothelin-B is located in exon 2, whereas transcription and translation of the previously described smoothelin-A starts inside exon 10. Smoothelin-A and -B were demonstrated to be generated by two physically separated promoters. Splice variants within the calponin homology domain at the 3' end of the gene were found for both isoforms. Conclusions: Two major smoothelin isoforms are generated from a single gene by a dual promoter system in a tissue specific manner. Further variation in the smoothelin proteins is achieved by alternative splicing in the calponin homology domain.


http://www.sciencedirect.com/science/article/B6T14-436F05F-8/2/bb7433bf5a271f7d092d1c5e3b79c9a9

Objective: Cardiotrophic growth factors with anti-cell death actions on cardiac myocytes have gained attention for treatment of patients with myocardial infarction. Hepatocyte growth factor (HGF) plays a role in tissue repair and protection from injuries, however, the physiological role of HGF in the myocardium has not been well defined. We asked if HGF would afford to the infarcted myocardium. Methods and results: Mature cardiac myocytes prepared from adult rats expressed barely detectable levels of the c-Met/HGF receptor, however, c-Met receptor expression increased during cultivation, which meant that cardiac myocytes are potential targets of HGF. Addition of hydrogen peroxide remarkably decreased the number of viable mature cardiac myocytes in primary culture, whereas treatment with HGF enhanced survival of the cells subjected to the oxidant stress. Although very low levels of c-Met/HGF receptor and HGF mRNA expression were seen in normal rat hearts, both c-Met/HGF receptor and HGF mRNA levels rapidly increased to much higher levels than normal, when the rats were subjected to myocardial infarction. Immunohistochemical analysis of the c-Met receptor indicated that this receptor was expressed in cardiomyocytes localized in the border regions of the viable myocardium and in non-infarcted regions following myocardial infarction. Conclusion: The c-Met/HGF receptor is induced in cardiomyocytes following myocardial infarction and HGF exhibits protective effect on
cardiomyocytes against oxidative stress. Our working hypothesis is that HGF may afford myocardial protection from myocardial infarction.


ObjectiveDefects in myocardial mitochondrial structure and function have been associated with heart failure in humans and animal models. Mice lacking the muscle LIM protein (MLP) develop morphological and clinical signs resembling human dilated cardiomyopathy and heart failure. We tested the hypothesis that defects in the cytoskeleton lead to dilated cardiomyopathy through mitochondrial dysfunction in the MLP mouse model.Methods and resultsOxidative phosphorylation activity was determined in left ventricles of MLP knockout (KO) mice and control littermates by measuring complex activities of the electron transport chain (I-IV) and ATP synthase (complex V). All complexes and citrate synthase (CS) showed decreased activities in the KO mice, although activity per amount of CS, a measure for mitochondrial density, was normal. Light and electron microscopy revealed a disorganization of mitochondria and a dramatic decrease in mitochondrial density, even revealing regions completely lacking mitochondria in the KO hearts. Real-time PCR analysis showed decreased transcript levels of mtDNA and nuclear encoded mitochondrial genes and of peroxisome proliferator activated receptor gamma co-activator 1[alpha] (PGC-1[alpha]), a key regulator of mitochondrial biogenesis. MtDNA copy number (ratio mtDNA/nuclear DNA) was slightly increased in the MLP KO mice.

ConclusionOur results show that the absence of MLP causes a local loss of mitochondria. We hypothesize that this is caused by a disturbed interaction between cytoskeleton and mitochondria, which interferes with energy sensing and energy transfer. Recovery of energy depletion by stimulating mitochondrial biogenesis might be a useful therapeutic strategy for improving the energy imbalance in heart failure.


Objective: To test the hypothesis that Vegf-B contributes to the pulmonary vascular remodelling, and the associated pulmonary hypertension, induced by exposure of mice to chronic hypoxia.

Methods: Right ventricular systolic pressure, the ratio of right ventricle/[left ventricle+septum] (RV/[LV+S]) and the thickness of the media (relative to vessel diameter) of intralobar pulmonary arteries (o.d. 50-150 and 151-420 [μm]) were determined in Vegfb knockout mice (Vegfb-/-; n=17) and corresponding wild-type mice (Vegfb+/+; n=17) exposed to chronic hypoxia (10% oxygen) or housed in room air (normoxia) for 4 weeks. Results: In Vegfb+/+ mice hypoxia caused (i) pulmonary hypertension (a 70% increase in right ventricular systolic pressure compared with normoxic Vegfb+/+ mice; PPPVegfb-/- mice hypoxia did not cause any increase in either right ventricular systolic pressure or pulmonary arterial medial thickness; also right ventricular hypertrophy (41% increase in RV/[LV+S]; PJPVegfb+/+ mice. Conclusion: Vegf-B may have a role in the development of chronic hypoxic pulmonary hypertension in mice by contributing to pulmonary vascular remodelling. If so, the effect of Vegf-B appears to be different from that of Vegf-A which is reported to protect against, rather than contribute to, hypoxia-induced pulmonary vascular remodelling.
Objective: The aim was to analyze the early postnatal changes in myocardial density, subsarcolemmal localization and isoform expression of dihydropyridine receptors in rat ventricle and the influence of thyroid status on these changes. Methods: Newborn rats were treated from postnatal day 2 with L-triiodothyronine (T3) or 6-n-propyl-2-thiouracil (PTU) and ventricles were collected on day 1, 7 and 14. Radioligand binding and cell fractionation (density gradient centrifugation) techniques were used to determine the tissue density of various receptors and their subcellular localization. To analyze dihydropyridine receptor [alpha]1 subunit isoform expression, cDNA fragments corresponding to a large portion of motif IV were amplified by reverse transcriptase-polymerase chain reaction and treated with appropriate restriction endonucleases to determine the frequency of splicing events at the level of motif IV. Results: The myocardial density of dihydropyridine receptors increased 3-fold from day 1 to day 14 in control rats, and this increase occurred predominantly in membrane entities equilibrating at high densities in sucrose gradient, that is, presumably, in junctional structures (dyadic couplings). This maturation was delayed after PTU-treatment, and somewhat accelerated by excess T3. The proportion of mRNA variants typical of foetal heart (IVS3A variant and 'deleted' variant, showing a 33-nucleotide deletion at the level of the extracellular loop between IVS3 and IVS4) decreased with age in control rats. This reduction was delayed after treatment with PTU but was not influenced by excess T3. Conclusion: Hypothyroidism impaired the early postnatal maturation of dihydropyridine receptors as regards both their concentration into junctional structures and the decrease in the relative expression of [alpha]1-subunit mRNA variants typical of foetal heart.

Cell Calcium (5)


Ca2+ homeostasis mechanisms, in which the Ca2+ entry pathways play a key role, are critically involved in both normal function and cancerous transformation of prostate epithelial cells. Here, using the lymph node carcinoma of the prostate (LNCaP) cell line as a major experimental model, we characterize prostate-specific store-operated Ca2+ channels (SOCs)--a primary Ca2+ entry pathway for non-excitable cells--for the first time. We show that prostate-specific SOCs share major store-dependent, kinetic, permeation, inwardly rectifying, and pharmacological (including dual, potentiation/inhibition concentration-dependent sensitivity to 2-APB) properties with "classical" Ca2+ release-activated Ca2+ channels (CRAC), but have a higher single channel conductance (3.2 and 12 pS in Ca2+- and Na+-permeable modes, respectively). They are subject to feedback inhibition via Ca2+-dependent PKC, CaMK-II and CaM regulatory pathways and are functionally dependent on caveolae integrity. Caveolae also provide a scaffold for spatial co-
localization of SOCs with volume-regulated anion channels (VRAC) and their Ca2+-mediated interaction. The TRPC1 and TRPV6 members of the transient receptor potential (TRP) channel family are the most likely molecular candidates for the formation of prostate-specific endogenous SOCs. Differentiation of LNCaP cells to an androgen-insensitive, apoptotic-resistant neuroendocrine phenotype downregulates SOC current. We conclude that prostate-specific SOCs are important determinants in the transition to androgen-independent prostate cancer.


http://www.sciencedirect.com/science/article/B6WCC-4CPDG1S-3/2/b268108754a5fafa2499243f6f9d5cbd

In blood vessels, the ability to control vascular tone depends on extracellular calcium entry and the release of calcium from inositol 1,4,5-trisphosphate receptor (IP3R)-gated stores located in both the endothelial and smooth muscle cells of the vascular wall. Therefore, we examined mRNA expression and protein distribution of IP3R subtypes in intact aorta, basilar and mesenteric arteries of the rat. IP3R1 mRNA was predominantly expressed in all three arteries. Immunohistochemistry showed that IP3R1 was present in both the muscle and endothelial cell layers, while IP3R2 and IP3R3 were largely restricted to the endothelium. Weak expression of IP3R2 was observed in the smooth muscle of the basilar artery. Co-localisation studies of IP3R subtypes with known cellular elements showed no association of any of the three subtypes with the endothelial cell plasma membrane, but a close association between the subtypes and actin filaments was observed in all cell layers. IP3R2 was found to be present near the endothelial cell nucleus. We are the first to demonstrate differential IP3R subtype distribution between the cell layers of the intact vascular wall and hypothesise that this may underlie the diversity of IP3R-dependent responses, such as vasoconstriction, vasodilation and vasomotion, displayed by arteries.


http://www.sciencedirect.com/science/article/B6WCC-468TDB1-3/2/5cbfbed3ca0c3ec9484c274fbcf3a590

Calsequestrin (CSQ) is the major Ca2+ binding protein of the cardiac sarcoplasmic reticulum (SR). Transgenic mice overexpressing CSQ at the age of 7 weeks exhibit concentric cardiac hypertrophy, and by 13 weeks the condition progresses to dilated cardiomyopathy. The present study used a differential display analysis to identify genes whose expressions are modulated in the CSQ-overexpressing mouse hearts to provide information on the mechanism of transition from concentric cardiac hypertrophy to failure. Cardiac ankyrin repeat protein (CARP), glutathione peroxidase (Gpx1), and genes which participate in the formation of extracellular matrix including decorin, TSC-36, Magp2, Osf2, and SPARC are upregulated in CSQ mouse hearts at 7 and 13 weeks of age compared to those of non-transgenic littermates. In addition, two novel genes without sequence similarities to any known genes are upregulated in CSQ-overexpressing mouse hearts. Several genes are downregulated at 13 weeks, including SR Ca2+-ATPase (SERCA2) and adenine nucleotide translocase 1 (Ant1) genes. Further, a functionally yet unknown gene (NM_026586) previously identified in the mouse wolffian duct is dramatically downregulated in CSQ mice with dilated hearts. Thus, CARP, Gpx1, and genes encoding extracellular matrix proteins may participate in the development of cardiac hypertrophy and fibrosis, and changes in SERCA2, Ant1, and NM_026586 mRNA expression may be involved in transition from concentric
to dilated cardiac hypertrophy.


http://www.sciencedirect.com/science/article/B6WCC-4C83495-4J2/87f594ac240575957befe0e1f26be9be

It has been long known that neoplastic transformation is accompanied by a lowered requirement for extracellular Ca2+ for growth. The studies presented here demonstrate that human fibroblastic cell lines produce the two commonly found 'housekeeping' isoforms of the plasma membrane Ca2+-ATPase (PMCA), PMCA1 b and 4b, and that the expression of both is demonstrably lower in cell lines neoplastically transformed by SV40 than in the corresponding parental cell lines. Western blot analyses of lysates from control (GM00037) and SV40-transformed (GM00637) skin fibroblasts revealed a 138 kDa PMCA whose level was significantly lower in the SV40-transformed cells relative to either total cellular protein or [alpha]-tubulin. Similar analyses of plasma membrane preparations from control (WI-38) and SV40-transformed (WI-38VA13) lung fibroblasts revealed 3-4-fold lower levels of PMCA in the SV40-transformed cells. Competitive ELISAs performed on detergent solubilized plasma membrane preparations indicated at least 3-4-fold lower levels of PMCA in the SV40-transformed cell lines compared to controls. Reverse transcriptase coupled-PCR analyses showed that PMCA1 b and PMCA4b were the only isoforms expressed in all four cell lines. The PMCA4b mRNA level detected by Northern analysis also was substantially lower in SV40 transformed skin fibroblasts than in non-transformed fibroblasts. Quantitative RT-PCR analyses showed levels of PMCA1 b and 4b mRNAs to be 5 and 10-fold lower, respectively, in GM00637 than in GM00037 when the levels of PCR products were normalized to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA. These results demonstrate that the expression of these distinct PMCA genes is substantially lower in SV40 transformed human skin and lung fibroblasts and may be coordinately regulated in these cells.


http://www.sciencedirect.com/science/article/B6WCC-4C834C3-5K/2/a813c84524cb8ff4f121b7f4af077171

Microfluorometric measurements in Fura-2-loaded single cultured human vascular endothelial cells were used to characterize the intracellular calcium [Ca2+]i responses triggered by extracellular application of adenosine 5'-triphosphate (ATP) and other nucleotides. Application of ATP or uridine 5'-triphosphate (UTP) gave rise to dose-dependent elevations of [Ca22+]i in all the cells tested. At saturating concentrations of agonist, the [Ca2+]i response was biphasic, with an early peak and a sustained plateau. Unlike peak responses, the sustained Ca2+ plateau was sensitive to removal of Ca2+ from the external medium. Mn2+ quenching revealed the presence of Ca2+ influx during the agonist-induced calcium plateau. The agonist-evoked calcium plateau was inhibited in a dose-dependent manner by the Cl-channel blocker NPPB, by the divalent cation Ni2+ and by the imidazole antimycotic econazole. Previously, these compounds have been shown to block store-operated Ca2+ entry. The two phases of the agonist-evoked [Ca2+]i response were blocked by the specific phospholipase C inhibitor U-73122 and by intracellular injection of low molecular weight heparin, suggesting the involvement of IP3 sensitive intracellular Ca2+ stores. The pharmacological profile of the response, using different nucleotides and analogues, ATP = UTP> ADP = UDP, and no responses to P2x1 and P2Y1 agonists, suggested the involvement of P2Y2 receptors. The expression of mRNA for the P2Y2 receptor was detected by RT-PCR analysis. These results indicate that P2Y2 receptors linked to intracellular Ca2+...
mobilization are present in human vascular endothelial cells. The initial [Ca2+]i mobilization is followed by a phase of elevated [Ca2+]i influx.

Cell Transplantation  (2)


http://www.sciencedirect.com/science/article/B6T55-3Y2N0PP-7/2/96dca67548cf7f7ef544fcd0f0bf39c48

Expression of a fluorescent reporter gene has been studied using two alternate promoters to transcribe the green fluorescent protein gfp from Aequorea victoria. The human cytomegalovirus (CMV) enhancer/promoter or the human muscle-specific creatine kinase promoter (CKM) were inserted along with the gfp cDNA into a plasmid expression vector based on a modified adeno-associated virus genome. Naked plasmid DNA was injected into the hamstring muscle of mdx mice and gfp gene expression determined from frozen muscle sections taken at 4, 14, and 42 days postinjection. Fluorescence patterns obtained by photomicroscopy and quantitative fluorescence measurements indicated a near-linear increase in the accumulation of the gfp in skeletal muscle during the length of the study, with gfp expression at 42 days being roughly four times the values obtained at 4 days. The levels of expression of gfp from the CKM construct were consistently higher than for the CMV construct. The CKM promoter/expression vector combination demonstrates significant potential for simple, direct delivery and long-term, high-level expression of genes in skeletal muscle.


http://www.sciencedirect.com/science/article/B6T55-3YS88KD-14/2/a0d2ebcf01cb12a49e8dcf2578283e58

A future possibility for treatment of genetic diseases may be gene therapy using autologous cord blood (CB) stem/progenitor cells. This might require cryopreservation of CB stem/progenitor cells prior to purification, gene transduction, and ex vivo expansion of cells. To address this possibility, nonadherent low density T-lymphocyte depleted (NALT-) cells from fresh or cryopreserved cord blood were sorted for CD34+++ phenotype, transduced with a recombinant retroviral vector encoding Fanconi anemia complementation C (FACC) gene, and cells expanded ex vivo in suspension culture for 7 days with growth factors. The results demonstrate: 1) high recovery of viable cells after thawing; 2) high efficiency purification of CD34+++ cells from NALT- cells prior to and after cryopreservation; 3) high degree of expansion of nucleated cells and immature progenitors from CD34+++ cells before and after cryopreservation; 4) efficient transduction with stable integration and expression of newly introduced genes in cryopreserved and then sorted stem/progenitor cells, as detected prior to and after ex vivo expansion; and 5) high efficiency transduction of single isolated CD34+++ cells obtained from cryopreserved NALT- CB. This
information should be of value for future studies evaluating the use of cryopreserved cord blood for gene transfer/gene therapy.

Cellular Immunology (8)


Coccidioides posadasii is a soil fungus that causes coccidioidomycosis or Valley Fever in the endemic regions of the southwestern US and Central America. Persons with decreased T cells reactivity and immune deficiency are at increased risk of developing severe disseminated infection. Among different mouse strains, DBA/2 mice are relatively resistant to C. posadasii whereas BALB/c mice are highly susceptible, and this discrepancy has been attributed to the difference in the development and expression of their Th1 cellular response. Dendritic cells (DC) are the most potent antigen-presenting cells that are activated after taking up pathogens or pathogens-derived antigens and regulate the immune response in the host, including Th1 cellular response. However, the DC responses against C. posadasii are not characterized. In the present study, we cultured bone-marrow derived DC (BMDC) from BALB/c and DBA/2 mice and infected with C. posadasii arthroconidia. The activation of BMDC was characterized by studying expression of cell surface co-stimulatory molecules (CD11c, MHC class II, CD40, CD80, and CD86), expression of genes encoding Toll-like receptors and release of IL-12. We found that the BMDC from DBA/2 mice showed significant upregulation of Toll-like receptor-2 and 4 genes expression, secretion of IL-12 (p C. posadasii.


http://www.sciencedirect.com/science/article/B6WCF-49WMTYD-1/2/c8537d4e00f19e85b8547847caf2e5d3

Investigation into the mechanism of action of vaccine adjuvants provides opportunities to define basic immune principles underlying the induction of strong immune responses and insights useful for the rational development of subunit vaccines. A novel HIV vaccine composed of plasmid DNA-encoding p55 gag formulated with poly-lactide-co-glycolide microparticles (PLG) and cetly trimethyl ammonium bromide (CTAB) elicits both serum antibody titers and cytotoxic lymphocyte activity in mice at doses two orders of magnitude lower than those required for comparable response to plasmid DNA in saline. Using this model, we demonstrated the increase in potency requires the DNA to be complexed to the PLG-CTAB microparticles. Furthermore, the PLG-CTAB-DNA formulation increased the persistence of DNA at the injection site, recruited mononuclear phagocytes to the site of injection, and activated a population of antigen presenting cells. Intramuscular immunization with the PLG-CTAB-DNA complex induced antigen expression at both the injection site and the draining lymph node. These findings demonstrate that the PLG-CTAB-DNA formulation exhibits multiple mechanisms of immunopotention.

http://www.sciencedirect.com/science/article/B6WCF-473FVF8-2/2/b2085c9be8bc9f18fb0de1458e6aea5f

In a search for novel early T cell activation transcripts, we identified expressed sequence tags (ESTs) more abundantly expressed in normal human CD4+ T lymphocytes fully activated by a 5 h exposure to CD3 plus CD28 mAbs, compared to the same cells stimulated with either CD3 mAb or CD28 mAb alone. An EST was identified that hybridized with a 1.7 kb transcript expressed in activated T cells but was undetectable by Northern blot analysis in resting T cells or other normal tissues. The T cell transcript was maximally induced within 6 h and remained elevated for at least 47 h. Induction of the transcript was blocked by cyclosporin A, FK506, and dexamethasone but not by rapamycin. The transcript was polyadenylated but lacked an open reading. A BLAST search of the NCBI database revealed that the transcript shared identity with the recently reported human BIC proto-oncogene that encodes a noncoding mRNA (W. Tam, Gene 274 (2001) 157). Our data demonstrate that transcriptional activation of the BIC proto-oncogene is an early and sustained T cell activation event and suggest an important role for noncoding mRNA in T cell function.


http://www.sciencedirect.com/science/article/B6WCF-48063T1-2/2/52ca69dc9062a20236a90c7c35b51510

Lactoferrin, a glycoprotein present in milk, mucosal secretions and neutrophils contributes to host defense. We have previously shown that orally given milk lactoferrin (LF) mediates anti-infectious and anti-inflammatory activities in vivo. Moreover, we have shown that LF could inhibit the LPS-induced IL-6 secretion in a human monocytic cell line, THP-1. This observation was expanded in the present study investigating the capacity of LF to inhibit cytokine mRNA expression and the involvement of nuclear transcription factor kappa B (NF-[-kappa]B). Cells (THP-1 and Mono Mac 6 monocytic cell lines) were stimulated with Escherichia coli LPS (5-10 ng/106 cells) and LF was added (50-500 [mu]g/106 cells) 30 min before, or after the LPS addition. By a semiquantitative RT-PCR lower levels of TNF-[-alpha], IL-1[beta], IL-6, and IL-8 mRNA expression were detected at the peak of the expression in THP-1 cells treated with LF. The reduction in the cytokine expression was followed by a similar reduction in the secreted cytokines as analyzed by ELISA. LF down-regulated also the IL-10 secretion (detected only in LPS-stimulated Mono Mac 6 cells). A similar level of inhibition of these cytokines was detected regardless of the time at which LF was added to the cells in relation to LPS. In addition, LF was internalized into cells and detected in the nucleoli as determined by immunostaining and immunofluorescence. Moreover, by electrophoretic mobility shift assay (EMSA) analysis LF decreased the LPS-induced binding of NF-[-kappa]B to the TNF-[-alpha] promoter. The results show that LF down-regulates the LPS-induced cytokine production in monocytic cells. The inhibitory mechanism is suggested to involve the interference of LF with NF-[-kappa]B activation.

Iwamoto, S., M. Ishida, et al. "A human Langerhans cell-like cell line, ELD-1, promotes CD8 T cells to produce IFN-[-gamma] through CD70-dependent alternative pathway." Cellular Immunology In
A novel pathway of CD8+ T-cell activation by a previously established human Langerhans cell (LC)-like cell line, ELD-1 [Dendritic Cells 9 (1999) 41] is reported. ELD-1 cells possess LC-specific and dendritic cell (DC) lineage-specific markers including Birbeck granules. Intriguingly, ELD-1 cells stimulated interferon (IFN)-[gamma] production by purified allogeneic CD8+ T cells in an IL-2- but not IL-12-dependent manner, but failed to stimulate CD4+ T cells due to their lack of HLA-DR, CD40, CD80, and CD86 expression. Comparing active and inactive subclones of ELD-1 cells revealed that CD70 was a key molecule determining stimulatory ability. This was confirmed by the ability of transfected CD70-encoding cDNA to confer stimulatory capacity on inactive subclones of ELD-1. Therefore, it is concluded that CD70 expressed on ELD-1 cells has a crucial role in stimulating IFN-[gamma] production by CD8+ T cells through an alternative pathway which does not require CD4+ T-cell help or CD28-B7 interactions.


http://www.sciencedirect.com/science/article/B6WCF-4DKSWY1-C7/2/260cd5411728e0a060fcd31ba6b0b49

Although murine peritoneal B cells were homogenously positive for an epitope Lp-2, coded for by the alternative exon 4 of the CD45 gene, they were heterogenous with respect to the expression of another CD45R epitope, 6B2, of unknown exon dependency. While the majority of 6B2-high peritoneal B cells was composed of CD5- B cells, those with low or negative 6B2 were CD5+ B cells. Both 6B2+ and 6B2- peritoneal B cells expressed mainly the same largest CD45R transcripts, with all three alternative exon (4, 5, and 6) sequences. Further, a CD5+ B lymphoma cell line, BCL-1, which was found to be Lp-2+6B2- also had the largest isoform of CD45R molecules with all three alternate structures. Although enzyme digestion studies suggested that the 6B2 epitope resides in protein, not in sugar structures, it is likely that a post-translational modification of CD45R molecules is responsible for the presence or absence of 6B2 epitope expression on peritoneal CD5+ B cells. This event may be related to the differential role of CD45R molecules in regulating lymphocyte function.


http://www.sciencedirect.com/science/article/B6WCF-4F0KJ94-2/2/16a796b06388f7a13ea2fddad3187cd27

The chemokine receptor CX3CR1 is thought to regulate inflammation in part by modulating NK cell adhesion, migration, and killing in response to its ligand CX3CL1 (fractalkine). Recent reports indicate that IL-15, which is essential for development and survival of NK cells, may negatively regulate CX3CR1 expression, however, the effects of the cytokine on human NK cell CX3CR1 expression and function have not been fully delineated. Here, we demonstrate that short term culture in IL-15 decreases surface expression of CX3CR1 on cultured CD56+ cells from human blood resulting in diminished chemotaxis and calcium flux in response to CX3CL1. Cells cultured long term in IL-15 (more than five days) completely lost surface expression as well as mRNA and protein for CX3CR1. The effect was specific since mRNA for CCR5 was increased and mRNA for CXCR4 was unchanged in these cells by IL-15. Thus, exogenous IL-15 is a negative regulator of
CX3CR1 expression and function in human CD56+ NK cells. The data imply that the use of IL-15 alone to expand NK cells ex vivo for immunotherapy may produce cells impaired in their ability to traffic to sites of inflammation.


http://www.sciencedirect.com/science/article/B6WCF-471VF7J-12/2/699d2488c46084f5c1dffd217e9aff1f

In vitro 5-day cultures of naive spleen cells with viable Listeria monocytogenes (VLM), but not heat-killed L. monocytogenes, induced CD4+ T cells that produced IFN-[gamma] upon secondary antigen stimulation. The VLM-induced Listeria-specific T cells produced IFN-[gamma] but lacked expression of IL-2 and IL-4. To study the role of IFN-[gamma] in the induction of the IFN-[gamma]-producing T cells, we added anti-IFN-[gamma] mAb to the primary culture and analyzed IFN-[gamma] production upon secondary antigen stimulation. Addition of anti-IFN-[gamma] mAb to the culture suppressed generation of IFN-[gamma]-producing CD4+ T cells, suggesting that IFN-[gamma] is important in the induction of IFN-[gamma]-producing CD4+ T cells. Furthermore, our results showed that depletion of NK cells from spleen cells by anti-asialo GM1 antibody plus complement before culture enhanced induction of IFN-[gamma]-producing CD4+ T cells. Although NK cells are known to produce IFN-[gamma], the results indicate that NK cell-derived IFN-[gamma] may not be important in induction of the Listeria-specific IFN-[gamma]-producing CD4+ T cells in the culture system. In addition, we demonstrated that IFN-[gamma] expression was high in CD4+ T cells from cultures of spleen cells with VLM at the primary culture level. These results suggest that IFN-[gamma] derived from T cells may enhance production of IFN-[gamma] by CD4+ T cells, while NK cells rather suppress the induction of IFN-[gamma]-producing CD4+ T cells.

Cellular Signalling  (8)


http://www.sciencedirect.com/science/article/B6T2M-4F8TKK-3/2/e67a784a06fe21d076165f6c914235db

Sphingosine-1-phosphate, a lipid mediator produced by sphingosine kinases, regulates diverse cellular processes, ranging from cell growth and survival to effector functions, such as proinflammatory mediator synthesis. Using human A549 epithelial lung carcinoma cells as a model system, we observed transient upregulation of sphingosine kinase type 1 (SPHK1) enzyme activity upon stimulation with both TNF-[alpha] or IL-1[beta]. This transient activation of SPHK1 was found to be required for cytokine-induced COX-2 transcription and PGE2 production, since not only specific siRNA (abolishing both basal and induced SPHK1 enzyme activity), but also a dominant-negative SPHK1 mutant (suppressing induced SPHK1 activity only) both reduced COX-2 and PGE2. Furthermore, TNF-[alpha]- or IL-1[beta]-induced transcription of selected cytokines, chemokines, and adhesion molecules (IL-6, RANTES, MCP-1, and VCAM-1) was found to
require SPHK1 activation. Suppression of SPHK1 activation led to reduction of cytokine-induced [kappa][alpha] phosphorylation and consequently diminished NF[kappa][B] activity due to reduced nuclear translocation of RelA (p65), explaining the dependence of inflammatory mediator production on SPHK1 activation. Inhibition of basal SPHK1 activity by N,N-dimethylphosphinosine or by downregulation of its expression using siRNA induced spontaneous apoptosis in A549 cells, an effect that can be explained through interference with constitutive NF[kappa][B] activity in this cell type. In contrast, expression of the dominant-negative mutant did not induce apoptosis. Taken together, these findings demonstrate a role of SPHK1 activation in proinflammatory signalling and of SPHK1 basal activity in survival of A549 lung carcinoma cells.


S49 cells expressed type 2 somatostatin receptors (sstr2) by immunoblotting. Analysis by reverse transcription and polymerase chain reaction (RT-PCR) methodologies showed that S49 cells express predominantly sstr2A and sstr2B mRNAs; other subtypes were either not detected, in the case of sstr1, sstr3, sstr4, or variably detected, in the case of sstr5. No mutations were present in S49 cells at codon 12, 13, or 61 of the N-, K-, or H-ras genes. Nevertheless, randomly growing S49 cells contained Raf-1 activity by specific immune complex kinase assays. Treatment of S49 cells with somatostatin transiently inactivated the basal activity of Raf-1, but not that of B-Raf. Addition of somatostatin plus guanylyl-5'-yl imidodiphosphate (GMPPNP) to S49 membranes stimulated PTPase activity. The concentration dependence for stimulation of PTPase activity correlated with high affinity binding of [125I-Tyr11]somatostatin-14. Both the effect of somatostatin to stimulate PTPase activity and to inactivate Raf-1 were abrogated by PTx. PTPase activity stimulated by somatostatin plus GMPPNP was recovered in a peak of high apparent Mr (670,000) after solubilisation with Triton X-100 and Superose 6 chromatography. Furthermore, addition of activated, brain G[alpha]i/o subunits to fractions from control membranes stimulated PTPase activity in the high Mr peak. Thus, S49 membranes contain a G-protein regulated PTPase (PTPase-G), and PTPase-G in these cells may reside in a high molecular weight complex.


Lipopolysaccharide (LPS) induces a delayed release (lag phase of 2-4 h) of arachidonic acid (AA) and prostaglandins in liver macrophages. Group IV cytosolic phospholipase A2 (cPLA2) becomes phosphorylated within minutes after the addition of LPS. The phosphorylated form of cPLA2 shows an enhanced in vitro activity. The Ca2+ dependence of cPLA2 activity is not affected by phosphorylation of the enzyme. In addition, LPS induces an enhanced expression of cPLA2 mRNA (after 2-4 h) and an enhanced expression of cPLA2 protein (after 8 h). The cellular cPLA2 activity is enhanced about twofold 24 h after LPS treatment. Liver macrophages constitutively express mRNAs encoding Groups V and IIA secretory PLA2 (sPLA2). LPS has no effect on the levels of Groups V and IIA sPLA2 mRNA expression. Despite mRNA expression, Groups V and IIA sPLA2 protein and sPLA2 activity are not detectable in unstimulated or LPS-
stimulated liver macrophages. Collectively, these and earlier [Mediators Inflammation 8 (1999) 295.] results suggest that in liver macrophages the LPS-induced delayed release of AA and prostanoids is mediated by phosphorylation and an enhanced expression of cPLA2, a de novo expression of cyclooxygenase (COX)-2, but not by the actions of Group V or Group IIA sPLA2.


http://www.sciencedirect.com/science/article/B6T2M-45TS5Y3-4/2/97d2301fd3edc1036bad353186cc3c2d

Activation regulates the responsiveness of G-protein-coupled receptors (GPCRs) on T cells, and modifications in the activity of GPCRs characterize lymphocytes from some immune disorders such as multiple sclerosis (MS) and rheumatoid arthritis (RA). Some lines of evidence suggest that such an effect is connected with the altered expression of some GPCRs regulatory proteins. Herein we demonstrate that phitoemagglutinin (PHA)-induced activation leads to differential expression of G-protein-coupled receptor kinase (GRK) 2, GRK3, [beta]-arrestin-1, regulators of G-protein signalling (RGS) 2, and RGS16 and decreases responsiveness of mononuclear leukocytes (MNL) to the [beta]-adrenergic agonist isoproterenol. Interferon beta-1a (IFN[beta]-1a), which is known to ameliorate the course of MS, counteracts the activation-induced effects on the expression of these GPCR regulatory proteins in MNL. Furthermore, IFN[beta]-1a quenches the effects of PHA on the isoproterenol-induced accumulation of cyclic AMP (cAMP). We suggest that regulation of GPCRs responsiveness may be a relevant property of IFN[beta]-1a in MS.


http://www.sciencedirect.com/science/article/B6T2M-3X6SDJX-7/2/0dd29e9a57f5729118054e8194b0f2a

67% of total cAMP phosphodiesterase activity (PDE) in cultured rat hepatocytes could be detected in the cytosol, 15% in plasma membrane, 15% in 'dense vesicle,' and 3% in endoplasmatic reticulum fractions. Up to 84% of the PDE activity of the cytosol is represented by the rolipram-sensitive PDE 4. ICI 118233-inhibited PDE 3 was found predominantly in membranes. We were able to show that dexamethasone acts on the PDE 4 in cytosolic and plasma membrane fractions whereas glucagon effected the PDE 4 of the cytosol and the PDE 3 in 'dense vesicle' membranes. Primary culture of hepatocytes was used to study long-term effects of dexamethasone and glucagon on PDE 4 activity. Addition of dexamethasone (0.1 [mu]M) at the beginning of cultivation leads to a decrease of total PDE 4 activity whereas after 24 h precultivation no dexamethasone effect could be observed. Glucagon effects on PDE 4 were investigated in 20 h precultured hepatocytes. Maximal stimulation was achieved after 2 h of exposure. PDE 4 subtypes A, B, D and, to a lesser degree, subtype C could be detected by RT-PCR analysis. The results of semiquantitative RT-PCR show that the presence of dexamethasone during the first 24 h of cultivation reduced selectively the transcription of PDE 4D, whereas glucagon was without any effect. Also the translation of PDE 4D was reduced as shown in the Western blot. We would like to discuss the way that dexamethasone influences PDE 4D expression--most likely in combination with other factors such as cytokines--during the time of cell plating, whereas glucagon actions are part of metabolic regulations via phosphorylation reactions.

http://www.sciencedirect.com/science/article/B6T2M-47PGCMJ-8/2/bee22a6a229f3f7d93107470aed975da

Five high affinity G-protein-coupled receptors for sphingosine 1-phosphate (S1P) have been characterised so far (S1P1,2,3,4,5 formerly referred to as edg1,5,3,6,8). In this study, we show that S1P, dihydro-sphingosine 1-phosphate (dihydro-S1P) and dioleoylphosphatidic acid (doPA) are agonists for the orphan receptor GPR63. All three phospholipids mobilise intracellular calcium in CHO cells transiently transfected with GPR63. Calcium signals required cotransfection of a chimeric G[alpha]q/i protein in a fluorometric imaging plate reader (FLIPR(TM)) assay but did not require overexpressed G proteins in an aequorin assay, using a green fluorescent protein (GFP)-aequorin fusion protein as a bioluminescent Ca2+ reporter. GPR63 expression in CHO cells confers proliferative responses to S1P in a pertussis toxin (PTX)-insensitive manner. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) indicated highest expression in brain, especially in the thalamus and the nucleus caudatus. In peripheral tissues, highest expression was observed in thymus, stomach and small intestine; lower abundance of transcripts was detected in kidney, spleen, pancreas and heart. The discovery that S1P, dihydro-S1P and dioleoylphosphatidic acid activate GPR63 will facilitate the identification of agonists and antagonists, and help to unravel the biological function of this receptor.


http://www.sciencedirect.com/science/article/B6T2M-3WHKSRF-G/2/ceeb21c87f8c2aeae04404e1fe3849be

Members of the type 2 phosphatidic acid phosphatase (PAP2) family catalyse the dephosphorylation of phosphatidic acid (PA), lysophosphatidate and sphingosine 1-phosphate. Here, we demonstrate the presence of a Mg2+-independent and N-ethymaleimide-insensitive PAP2 activity in cultured guinea-pig airway smooth muscle (ASM) cells. Two PAP2 cDNAs of 923 and 926 base pairs were identified and subsequently cloned from these cells. The ORF of the 923 base pair cDNA encoded a protein of 285 amino acids (Mr = 32.1 kDa), which had 94% homology with human PAP2a (hPAP2a) and which probably represents a guinea-pig specific PAP2a (gpPAP2a1). The ORF of the 926 base pair cDNA encoded a protein of 286 amino acids (Mr = 32.1 kDa) which had 84% and 91% homology with hPAP2a and gpPAP2a1, respectively. This protein, termed gpPAP2a2, has two regions (aa 21-33 and 51-74) of marked divergence and altered hydrophobicity compared with hPAP2a and gpPAP2a1. This occurs in the predicted first and second transmembrane domains and at the extremes of the first outer loop. Other significant differences between gpPAP2a1/2 and hPAP2a, hPAP2b and hPAP2c occur at the cytoplasmic C-terminal. Transient expression of gpPAP2a2 in Cos-7 cells resulted in an approx. 4-fold increase in Mg2+-independent PAP activity, thereby confirming that gpPAP2a2 is another catalytically active member of an extended PAP2 family.

The cytoplasmic domain of the platelet-derived growth factor (PDGF) [beta]-receptor was expressed in insect cells by using a baculovirus system. The resulting protein was a constitutively active tyrosine kinase that could phosphorylate both protein and peptide substrates. A recently identified potent and selective inhibitor of intact PDGF receptor autophosphorylation, 3744W, inhibited the autophosphorylation of the cytoplasmic domain both in vitro (50 1.8 +/- 0.12 [mu]M) and within intact insect cells (50 2.0 [mu]M). However, under identical assay conditions, 3744W did not inhibit the phosphorylation of the synthetic polymeric peptide poly(Glu4Tyr1) even at concentrations as high as 100 [mu]M. These results suggest that, although 3744W inhibits PDGF receptor autophosphorylation directly, it can discriminate between phosphate acceptor substrates.

**Chem Senses (2)**


http://chemse.oupjournals.org/cgi/content/abstract/27/4/319

A taste tissue cDNA library of the fleshfly Boettcherisca peregrina was screened with a subtracted cDNA probe enriched with taste-receptor-tissue-specific cDNA. Seven genes were identified with sequence similarity to insect odorant-binding protein (OBP) genes. The predicted amino acid sequences of the genes contain the putative signal peptide sequence at the N-terminal and most of them conserve the six cysteines common to known insect OBPs. These genes show a high degree of sequence divergence with [-]20% amino acid identity. The most striking feature was that all seven of these genes are expressed mainly in the taste tissues, such as the labellum and tarsus, unlike the known insect OBP genes expressed in olfactory tissue. The predicted amino acid sequences had the highest degree of sequence similarity to the Drosophila melanogaster OBPs named pheromone binding protein-related proteins (PBPRPs). These gene products are here referred to as gustatory PBP-related proteins (GPBPRPs) 1-7. Homologous GPBPRP genes were found also in D. melanogaster by database search and are shown to be expressed in Drosophila taste tissues.


http://chemse.oupjournals.org/cgi/content/abstract/27/1/13

Systemic or topical application of glucocorticoid is the treatment of choice for olfactory disturbance. Recently, Na+/K+ ATPase and glucocorticoid receptor immunoreactivity in the olfactory mucosa was reported. To elucidate a glucocorticoid action on Na+/K+ ATPase production, an animal model was produced by an intra-nasal application of 5% ZnSO4 solution to Wistar rats. Dexamethasone was injected i.p. (0.01 mg/100 g) for 14 days after the insult. Histologically, the regeneration process was completed on day 14 in both dexamethasone- and
saline-injected control rats. We used a quantitative polymerase chain reaction (PCR) method to evaluate mRNA production of Na+/K+ ATPase and glucocorticoid receptor. In dexamethasone-injected rats, up-regulation of glucocorticoid receptor mRNA (95% more than control rats, P = 0.00068, unpaired t-test) and of Na+/K+ ATPase mRNA expression (76% more than control rats, P = 0.0042) was observed on day 14. The increased Na+/K+ ATPase expression in the regenerated olfactory mucosa is thought to be beneficial for an active uptake of K+, which is released during excitation, around olfactory neurons and for the transepithelial absorption of Na+ from olfactory mucus. Dexamethasone may thus contribute to the recovery of function after the morphological regeneration in part, at least, through its receptor by regulation of the ionic concentration in the olfactory mucosal microenvironment.

Chemico-Biological Interactions (9)


http://www.sciencedirect.com/science/article/B6T56-3XK16HH-N/2/2dc376d52b7d824216bf6a90d4502804

The flavin-containing monooxygenase (FMO, EC 1.14.13.8) is involved in the metabolism of a number of important xenobiotics including many which affect the central nervous system (CNS). Recently, reports in the literature concerning the amount, activity, location, and isozyme characteristics of this enzyme in the brain have presented conflicting evidence. In order to resolve some of the controversy surrounding FMO in the brain, a highly sensitive method for the detection of flavin-containing monooxygenase (FMO) mRNA in whole brain was employed. A poorly conserved region of FMO transcripts was used to design five sets of oligonucleotide primers. Each primer set was specific for one of the five currently known isoforms of FMO. Four and five isoforms, respectively, are expressed in rabbit liver and kidney, as determined by reverse transcription-polymerase chain reaction. However, only one set of primers amplified a specific rabbit brain cDNA fragment. The sequence of the amplification product affirmed its identity as a segment of FMO4 cDNA. Thus, the FMO of rabbit brain may consist of a single, as yet uncharacterized isozyme and, contrary to several recent reports, is likely to be expressed at low levels.


http://www.sciencedirect.com/science/article/B6T56-3VWP2MP-4/2/0985441f9ee5173c4c5d2afa64897727

During studies designed to subclone human phenol sulfotransferase (STP and STM) sequences for use in heterologous E. coli-based expression systems, we designed two oligonucleotide primers that would allow for the simultaneous PCR amplification of expression cassettes containing the coding regions of the STP1, STP2 and STM cDNAs. Following total RNA isolation from human liver, reverse transcription of cDNA, PCR amplification under standard conditions, plasmid subcloning and restriction analysis to select for suitable ST recombinants, we recovered
plasmids containing inserts corresponding to STP1, STP2 and STM. However, ten additional, closely related but apparently novel ST sequences were also isolated. Alignments of the three known ST sequences (and one published allelic variant) with these new clones revealed that each one appears to be a PCR-generated modular chimera possessing a combination of DNA segments derived from STP1, STP2 and STM. This observation should serve as an alert to the potential pitfalls of using PCR techniques for the cloning of highly related genes and their cDNA products, especially when PCR primer design allows for the amplification of multiple products in a single reaction.


http://www.sciencedirect.com/science/article/B6T56-3T149TG-9/2/ec735a60edaee6f3f929ae662e3ccea4

We recently reported the cloning of full-length cDNAs corresponding to mRNAs of three GST-[pi] genes, hGSTP1*A, hGSTP1*B and hGSTP1*C, as well as, the isolation of the full-length hGSTP1*C, of the human glutathione S-transferase-[pi] (GST-[pi]) gene that is characterized by a A->G transition at +1404 in exon 5 and a C->T transition at +2294 in exon 6. Although the promoter of the isolated gene was identical to that of the previously described GST-[pi] gene isolated from the MCF 7 and the HPB-ALL cell lines, both of which were hGSTP1*A, a number of structural differences were observed, including, nucleotide transitions, transversions, deletions and insertions, some of which created new restriction enzyme cleavage sites. A guanine insertion in the insulin response element, IRE, in intron 1 created an additional site for 5'-cytosine methylation. Seven repeat retinoic acid response element (RARE) consensus half sites, A(G)GG(T)TC(G)A at +1521 to +1644 were identified in the cloned hGSTP1*C. Five of the RARE half-sites had the minimal spacer nucleotide requirement for functionality and DNA mobility shift analysis with different pairs of the RARE half-sites and supershift studies using antibodies against RAR-[beta] showed significant binding of nuclear protein complexes from RA-treated cells to these RAREs. GST-[pi] gene expression was increased significantly in cells transfected with the GST-[pi] gene and treated with all-trans RA. These results contrast with those in a previous report in which RA was shown to suppress the GST-[pi] promoter, and indicate a complex mechanism of RA-mediated GST-[pi] gene regulation in tumor cells.


http://www.sciencedirect.com/science/article/B6T56-4DX276P-1/2/975093134a2588904aa294cdcf2e02b

Xenobiotics, including drugs, can influence cytochrome P450 (CYP) activity by upregulating the transcription of CYP genes. To minimize potential drug interactions, it is important to ascertain whether a compound will be an inducer of CYP enzymes early in the development of new therapeutic agents. In vivo and in vitro studies are reported that demonstrate the use of liver and intestinal slices as an in vitro model to predict potential CYP induction in vivo. Rat liver slices and intestinal slices were incubated, for 24 h and 6 h, respectively, with [beta]-naphthoflavone ([beta]NF), phenobarbital (PB) or dexamethasone (DEX). In an in vivo study, rats were treated with the same compounds for 3 days. In vivo and in vitro CYP mRNA levels were measured by using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). In addition, CYP enzyme activities were determined in rat liver slices after 48 h incubation. In both rat liver and intestinal slices, [beta]NF significantly induced CYP1A1, CYP1A2 and CYP2B1
mRNA levels. PB significantly induced CYP2B1. In liver slices a minor induction of CYP1A1 and CYP3A1 by PB was observed, whereas DEX significantly induced CYP3A1, CYP2B1 and CYP1A2 mRNA levels. The induction profiles (qualitative and quantitative) observed in vivo and in vitro are quite similar. All together, these data demonstrate that liver and intestinal slices are a useful and predictive tool to study CYP induction.


http://www.sciencedirect.com/science/article/B6T56-3T149TG-12/2/b45935a01db2c20aa59993dbdce1b205

Glutathione S-transferases (GSTs) are a family of isoenzymes involved in cellular detoxification. Previous studies have correlated the absence of the GSTM1 protein with an increased risk of developing some cancers, especially lung or bladder cancer, in heavy smokers. In this study, we determined GSTM1 gene polymorphisms in a French western population of 437 female controls and 361 community breast cancer patients. Three distinct alleles of this gene may be identified: GST M1* A allele, GST M1* B allele, and GST M1* 0 allele (which is deleted). Null patients (GSTM1 0) are homozygous for the deletion. We determined in our two populations, patients with no, one or two GSTM1 alleles. The comparative analysis of our two populations did not demonstrate any statistically significant difference in GSTM1 allelotype distribution between the two groups (P=0.43), although the null genotype was the more frequent in patients. The predominance of the null genotype was significant in the oldest group of patients (>55) (P=0.006), suggesting that GSTM1 null genotype may play an important role in breast cancer susceptibility in the elderly. This was not observed in the youngest age group, i.e. P=0.25), or in the patients aged from 40 to 55 years old (P=0.37). Our results also point out a putative protective role of the A allele in the older female control group (P=0.02), especially in subjects hemizygous for these alleles (P=0.03). A prospective study will be of interest to investigate the effect of dosage of the gene.


http://www.sciencedirect.com/science/article/B6T56-42SGGWV-20/2/9217ed08d3abd8667a72000f8471f3ff

Since aldose reductase is localized primarily in lens epithelial cells, osmotic insults induced by the accumulation of sugar alcohols occur first in these cells. To determine whether the accumulation of sugar alcohols can induce lens epithelial cell death, galactose-induced apoptosis has been investigated in dog lens epithelial cells. Dog lens epithelial cells were cultured in Dulbecco’s modified Eagle’s minimum essential medium (DMEM) supplemented with 20% fetal calf serum (FCS). After reaching confluence at fifth passage, the medium was replaced with the same DMEM medium containing 50 mM -galactose and the cells were cultured for an additional 2 weeks. Almost all of the cells cultured in galactose medium were stained positively for apoptosis with the terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick end labeling (TUNEL) technique. Agarose gel electrophoresis of these cells displayed obvious DNA fragmentation, known as a ladder formation. All of these apoptotic changes were absent in similar cells cultured in galactose medium containing 1 [mu]M of the aldose reductase inhibitor AL 1576. Addition of AL 1576 also reduced the cellular galactitol levels from 123+/−10 [mu]g/106 cells (n=5) to 3.9+/−1.9 [mu]g/106 cells (n=5). These observations confirm that galactose induced apoptosis occurs in dog lens epithelial cells. Furthermore, the prevention of apoptosis by an aldose reductase
inhibitor suggests that this apoptosis is linked to the accumulation of sugar alcohols.


http://www.sciencedirect.com/science/article/B6T56-3VWP2MP-P/2/16d9cf75aa5f4c4f511be0be13b7e9525

Three related forms of phenol sulfotransferase (PSULT), thermostable ST1A2 (SULT1A2hum) and ST1A3 (SULT1A1hum) and a thermolabile TL-PST (SULT1A3hum), are known to exist in human livers. Thermostable forms, whose activities are polymorphically distributed, have been shown to mediate the bioactivation of carcinogenic N-hydroxy arylamines and heterocyclic amines. To clarify the nature of the sulfation polymorphism, the study compared the expressed levels of ST1A2, ST1A3 and TL-PST mRNAs in human livers by the method of reverse-transcriptase polymerase chain reaction (RT-PCR), utilizing HindIII, BamHI and SnaBI sites which were unique to the above PSULT cDNAs, respectively. Of the PCR products derived from human liver (n=26), 43-89, p-nitrophenol and dopamine sulfation rates ranged from 440-2670 and p-nitrophenol and dopamine sulfations. Relative levels of hepatic ST1A3 mRNA were non-normally distributed and correlated significantly with p-nitrophenol sulfation. In addition, variant forms of ST1A3 mRNA encoding Arg213His and Met223Val were detected in human livers. With regard to Arg213His, 28 individuals who had homozygous 213Arg alleles, 15 individuals who were heterozygotes and nine homozygous 213His individuals were found by a newly established genotyping method among 52 human liver samples. Frequency of 223Val allele was apparently lower than that of 213His allele, as no homozygous 223Val individual and only three individuals who were heterozygotes (223Met/Val) were observed among 52 individuals. These results suggest that regulation of p-nitrophenol sulfation occurs at the level of gene transcription of ST1A3 which is the major transcript of the three PSULT mRNAs and that a polygenic basis for the apparent genetic polymorphism of sulfation was likely because of the existence of ST1A3 variants.


http://www.sciencedirect.com/science/article/B6T56-47PPD21-9/2/c0e3ca60f33282e0b786954ac41cb66d

Three cDNAs encoding carbonyl reductases (CHC1-3) have been isolated and expressed in bacterial cells. The recombinant enzymes were further characterized with respect to substrate specificity, inhibitor sensitivity and response to pyruvate. In addition, the expression of the mRNAs of CHCRs encoding in brain, liver and kidney was analyzed by RT-PCR. Searches of EST files revealed that orthologues of both CHCR3 and human CBR3 are expressed in variety of human cells and tissues.


http://www.sciencedirect.com/science/article/B6T56-42SGGWV-2P/2/ef67e5d4b4a55869a687e3f3cd439572
Carbonyl reductase (CR) is an enzyme which can catalyze the oxidoreduction of various carbonyl compounds in the presence of NAD(P)H. With the PCR method, using primers carrying the conserved nucleotide sequence among mammalian CRs, we isolated three different cDNAs (CHCR1, CHCR2 and CHCR3) which encode a unique carbonyl reductase from the Chinese hamster. The PCR products of CHCR1 and CHCR2 were clearly isolated with Bpu1102I, BspEI and Xmal restriction enzymes. The nucleotide-sequence of CHCR3 was completely different from those of CHCR1 and CHCR2. The predicted double-wound \([\beta][\alpha][\beta][\alpha]\)-structures of the CHCRs suggests the presence of a typical NADP\(^+\)-binding motif and is similar to the corresponding region of 3\([\alpha]\),20\([\beta]\)-hydroxysteroid dehydrogenase and mouse lung tetrameric carbonyl reductase. The deduced amino acid sequence of CHCR1 showed a high homology to CHCR2 (>96%) and the other mammalian CRs (>81%). However, CHCR3 showed a high homology to human CBR3 (>86%) and a relatively lower homology to the other CHCRs (2 and PGF2[\alpha]) from the analyses of enzymatic reaction products. The results of Western blotting and RT-PCR suggest these CHCRs have a tissue-dependent-distribution in the Chinese hamster.

Chemistry & Biology  (5)


http://www.sciencedirect.com/science/article/B6VRP-41PVY58-9/2/902f074a49b89dce5f348667d95a9f52

Background: Polyene macrolides are a class of large macrocyclic polyketides that interact with membrane sterols, having antibiotic activity against fungi but not bacteria. Their rings include a chromophore of 3-7 conjugated double bonds which constitute the distinct polyene structure. Pimaricin is an archetype polyene, important in the food industry as a preservative to prevent mould contamination of foods, produced by Streptomyces natalensis. We set out to clone, sequence and analyse the gene cluster responsible for the biosynthesis of this tetraene.

Results: A large cluster of 16 open reading frames spanning 84985 bp of the S. natalensis genome has been sequenced and found to encode 13 homologous sets of enzyme activities (modules) of a polyketide synthase (PKS) distributed within five giant multienzyme proteins (PIMS0-PIMS4). The total of 60 constituent active sites, 25 of them on a single enzyme (PIMS2), make this an exceptional multienzyme system. Eleven additional genes appear to govern modification of the polyketide-derived framework and export. Disruption of the genes encoding the PKS abolished pimaricin production.

Conclusions: The overall architecture of the PKS gene cluster responsible for the biosynthesis of the 26-membered polyene macrolide pimaricin has been determined. Eleven additional tailoring genes have been cloned and analysed. The availability of the PKS cluster will facilitate the generation of designer pimaricins by combinatorial biosynthesis approaches. This work represents the extensive description of a second polyene macrolide biosynthetic gene cluster after the one for the antifungal nystatin.

Background: The ansamycin class of antibiotics are produced by various Actinomycetes. Their carbon framework arises from the polyketide pathway via a polyketide synthase (PKS) that uses an unusual starter unit. Rifamycin (rif), produced by Amycolatopsis mediterranei, is the archetype ansamycin and it is medically important. Although its basic precursors (3-amino-5-hydroxy benzoic acid AHBA, and acetic and propionic acids) had been established, and several biosynthetic intermediates had been identified, very little was known about the origin of AHBA nor had the PKS and the various genes and enzymes that modify the initial intermediate been characterized.

Results: A set of 34 genes clustered around the rifK gene encoding AHBA synthase were defined by sequencing all but 5 kilobases (kb) of a 95 kb contiguous region of DNA from A. mediterranei. The involvement of some of the genes in the biosynthesis of rifamycin B was examined. At least five genes were shown to be essential for the synthesis of AHBA, five genes were determined to encode the modular type I PKS that uses AHBA as the starter unit, and 20 or more genes appear to govern modification of the polyketide-derived framework, and rifamycin resistance and export. Putative regulatory genes were also identified. Disruption of the PKS genes at the end of rifA abolished rifamycin B production and resulted in the formation of P8/1-OG, a known shunt product of rifamycin biosynthesis, whereas disruption of the orf6 and orf9 genes, which may encode deoxysugar biosynthesis enzymes, had no apparent effect.

Conclusions: Rifamycin production in A. mediterranei is governed by a single gene cluster consisting of structural, resistance and export, and regulatory genes. The genes characterized here could be modified to produce novel forms of the rifamycins that may be effective against rifamycin-resistant microorganisms.


Background: The temporal and spatial control of the transition from vegetative to parasitic growth is critical to any parasite, but is essential to the sessile parasitic plants. It has been proposed that this transition in Striga spp. is controlled simply by an exuded oxidase that converts host cell-surface phenols into benzoquinones which act as developmental signals that mediate the transition. An understanding of this mechanism may identify the critical molecular events that made possible the evolution of parasitism in plants.

Results: PoxA and PoxB are identified as the only apoplastic phenol oxidases in Striga asiatica seedlings, and the genes encoding them have been cloned and sequenced. These peroxidase enzymes are capable of oxidizing the 60 known inducing phenols into a small set of benzoquinones, and it is these quinones that induce parasitic development. Analysis of the reaction requirements and comparisons to host enzymes, however, lead us to argue that PoxA and PoxB are not necessary for host recognition.

Conclusions: A new model is proposed where constitutive production of an activated oxygen species (in the case of Striga, H2O2) mediates host recognition. This strategy would allow a parasite to exploit abundant host enzymes to produce the diffusible recognition signals by converting a standard host defense into a parasitic offense.

Background: The branched cyclic dodecylpeptide antibiotic bacitracin, produced by special strains of Bacillus, is synthesized nonribosomally by a large multienzyme complex composed of the three bacitracin synthetases BA1, BA2 and BA3. These enzymes activate and incorporate the constituent amino acids of bacitracin by a thiotemplate mechanism in a pathway driven by a protein template. The biochemical features of these enzymes have been studied intensively but little is known about the molecular organization of their genes.

Results: The entire bacitracin synthetase operon containing the genes bacA-bacC was cloned and sequenced, identifying a modular structure typical of peptide synthetases. The bacA gene product (BA1, 598 kDa) contains five modules, with an internal epimerization domain attached to the fourth; bacB encodes BA2 (297 kDa), and has two modules and a carboxy-terminal epimerization domain; bacC encodes BA3, five modules (723 kDa) with additional internal epimerization domains attached to the second and fourth. A carboxy-terminal putative thioesterase domain was also detected in BA3. A putative cyclization domain was found in BA1 that may be involved in thiazoline ring formation. The adenylation/thioester-binding domains of the first two BA1 modules were overproduced and the detected amino-acid specificity coincides with the first two amino acids in bacitracin. Disruption of chromosomal bacB resulted in a bacitracin-deficient mutant.

Conclusions: The genes encoding the bacitracin synthetases BA1, BA2 and BA3 are organized in an operon, the structure of which reflects the modular architecture expected of peptide synthetases. In addition, a putative thiazoline ring formation domain was identified in the BA1 gene.


http://www.sciencedirect.com/science/article/B6VRP-4319MWC-8/2/9ea24535bf980bcce717d017b6b879af

Background: Spinosad is a mixture of novel macrolide secondary metabolites produced by Saccharopolyspora spinosa. It is used in agriculture as a potent insect control agent with exceptional safety to non-target organisms. The cloning of the spinosyn biosynthetic gene cluster provides the starting materials for the molecular genetic manipulation of spinosad yields, and for the production of novel derivatives containing alterations in the polyketide core or in the attached sugars.

Results: We cloned the spinosad biosynthetic genes by molecular probing, complementation of blocked mutants, and cosmid walking, and sequenced an 80 kb region. We carried out gene disruptions of some of the genes and analyzed the mutants for product formation and for the bioconversion of intermediates in the spinosyn pathway. The spinosyn gene cluster contains five large open reading frames that encode a multifunctional, multi-subunit type I polyketide synthase (PKS). The PKS cluster is flanked on one side by genes involved in the biosynthesis of the amino sugar forosamine, in O-methylations of rhamnose, in sugar attachment to the polyketide, and in polyketide cross-bridging. Genes involved in the early common steps in the biosynthesis of forosamine and rhamnose, and genes dedicated to rhamnose biosynthesis, were not located in the 80 kb cluster.

Conclusions: Most of the S. spinosa genes involved in spinosyn biosynthesis are found in one 74 kb cluster, though it does not contain all of the genes required for the essential deoxysugars. Characterization of the clustered genes suggests that the spinosyns are synthesized largely by mechanisms similar to those used to assemble complex macrodides in other actinomycetes. However, there are several unusual genes in the spinosyn cluster that could encode enzymes that generate the most striking structural feature of these compounds, a tetracyclic polyketide aglycone nucleus.
Chemosphere  (3)


http://www.sciencedirect.com/science/article/B6V74-3VHNVS-4/2/ecd803857bdc82b4492e56bf5af7c636

Environmental pollutants can have deleterious effects on living organisms. At high concentrations, or at high activities, they can cause acute toxicity damaging cells, tissues and organs. Chronic toxification, on the other hand, can also cause serious damage from bio-accumulation. Plants, as biological indicators, can measure both the actual and the potential effects of pollutants, when they are used to measure effects of heavy metals. We have applied a system of "molecular fingerprinting" based on PCR (RAPD: Random Amplified Polymorphic DNA) to the evaluation of the genotoxic effects of heavy metals in order to estimate the environmental risk connected with their potential mutagenic effects in the model plant Arabidopsis thaliana, ecotype Columbia. Genomic DNA was utilised for RAPD analysis using random primers (10-mers). DNA from plants exposed to heavy metals solution displayed polymorphic bands which were not detectable in DNA of unexposed plants. The enhanced formation of RAPD polymorphisms was also observed in DNA of plant exposed in situ to an industrial pollution source. The comparison between "unexposed" and "exposed" genomes show that RAPD analysis can be used to evaluate how the environmental pollutants modify the structure of DNA in living organisms.


http://www.sciencedirect.com/science/article/B6V74-4BHCD8F-9/2/605a5ede2017d5b95e39d350347b8fd9

Cytochrome P450-dependent microsomal monooxygenase (P450) activity was measured in control and atrazine-exposed third instar midge larvae, Chironomus tentans. Significantly elevated O-demethylase activity was observed in gut homogenates taken from midges exposed to atrazine concentrations from 1 to 10 ppm for 90 h. No significant induction was observed at atrazine concentrations below 1 ppm. A region of a cytochrome P450 family 4 gene was amplified and sequenced from C. tentans larvae. Alignments of inferred amino acid sequences with other insect CYP4 gene homologues indicate a high degree of similarity. Northern blot analysis employing the CYP4 gene fragment as a probe showed an over-expression in C. tentans exposed to atrazine. The results support the previously identified inducibility of cytochrome P450-dependent activity and provide insight into the potential consequences of atrazine exposure to aquatic organisms.


http://www.sciencedirect.com/science/article/B6V74-44J69N3-1/2/ada0f8d5150b2f8aeeed9b9b49110220

http://www.chestjournal.org/cgi/content/abstract/125/5/1843

Study objective: We investigated the gene expression profiles of malignant pleural mesothelioma (MPM) specimens to identify novel genes that are potentially involved in the oncogenic transformation of human pleural cells. Design: Complementary DNA (cDNA) microarray transcriptional profiling studies of 10 MPM cell lines and 4 MPM primary tumor specimens were performed using hierarchic clustering. To confirm microarray data, we used real-time polymerase chain reaction and immunoblotting. Results: Cluster analysis differentiated among epithelial (E), sarcomatoid, and biphasic MPM variants. Expression profiling identified common overexpressed or underexpressed genes in MPM. Notably, matriptase messenger RNA was found to be overexpressed by 826-fold in E MPM, with protein expression subsequently confirmed by immunoblot analysis. This recently characterized trypsin-like serine protease has been implicated in tumor invasion and metastasis of E-derived cancers, but has not been described until now in MPM. We also identified other novel genes, such as insulin-like growth factor binding protein 5 and a cDNA clone similar to proteolipid MAL2. Conclusions: Thus, further large-scale profiling of MPM may elucidate previously unrecognized molecular mechanisms by identifying novel genes that are involved in malignant transformation. Our study has now found matriptase to be one of these mesothelioma-associated genes, with potential pathogenic and therapeutic significance.


http://www.chestjournal.org/cgi/content/abstract/125/1/63

Background: The finding that only 15 to 20% of cigarette smokers acquire COPD suggests that there is a genetic predisposition to the disease. Genetic polymorphism of the group-specific component of serum globulin (Gc-globulin), also known as vitamin-D-binding protein, is considered one of the candidates for the susceptibility to COPD. However, the role of Gc-globulin polymorphism in the development of COPD remains inconclusive. Study objectives: To determine whether Gc-globulin gene polymorphism plays a role in the development of COPD in the Japanese population, and whether it is associated with the physiologic deterioration in COPD, and its radiologically detectable correlates. Design: Association study. Subjects and methods: One hundred three patients with COPD and 88 healthy smokers sampled from the Japanese population were genotyped for Gc-globulin by the restriction fragment-length polymorphism method. Based on the results of the genotyping, we investigated the relationship between Gc-globulin polymorphism and a physiologic/radiologic indicator of lung function, namely, the annual decline of FEV1 (dFEV1) in 86 patients with COPD and 21 healthy smokers. Additionally, high-resolution CT parameters such as low-attenuation area percentage (LAA%) and average CT number (mean CT score) were measured in 85 patients with COPD. Results: There was an increased proportion of Gc*1F homozygotes in the patients with COPD (32%) compared with the healthy smokers (17%) [p = 0.01; odds ratio, 2.3; 95% confidence interval, 1.2 to 4.6]. Patients with COPD and the Gc*1F allele showed a larger dFEV1 (p = 0.01), higher frequency with LAA% > 60% (p = 0.01), and lower mean CT score than patients without this allele (p = 0.03). Conclusion: Gc-globulin polymorphism is significantly associated with susceptibility to COPD, and
also with the severity of the disease.


http://www.chestjournal.org/cgi/content/abstract/126/1/66

Study objective: To investigate the relationship of common single nucleotide polymorphisms (SNPs) of the {beta}2-adrenergic receptor (AR) gene at codons 16 and 27, and the intermediate phenotype of airways hyperresponsiveness. Design: A case-control study in 543 white men (152 case patients and 391 control subjects), who were nested in an ongoing longitudinal cohort. Setting: Subjects were selected from the Normative Aging Study, an ongoing longitudinal cohort of healthy aging. Participants: Case patients were defined as those having a positive response to methacholine challenge testing. Control subjects were selected among those who did not have a diagnosis of asthma and who had no response to methacholine. Results: There was a trend for an association of the Arg16 SNP genotype with airways hyperresponsiveness (odds ratio, 1.25; 95% confidence interval, 0.96 to 1.64 [in an additive model]). In stratified analyses, the effect of the Arg16 variant was seen mainly among nonsmokers. Smokers had increased risks for airway hyperresponsiveness regardless of genotype at either SNP. Using a program to estimate haplotype frequencies, three common haplotypes were identified. Adjusting for age, baseline FEV1, serum IgE level, and smoking status, the Gly16/Gln27 haplotype was negatively associated with airways hyperresponsiveness in the full complement of case patients and control subjects (score statistic, -2.43; p = 0.02). The effect of the {beta}2-AR haplotypes was much stronger among lifelong nonsmokers, among whom the Gly16/Gln27 haplotype remained negatively associated with airways hyperresponsiveness (score statistic, -3.114; p = 0.002), whereas the Arg16/Gln27 haplotype was positively associated with airways hyperresponsiveness (score statistic, 3.142; p = 0.002). No effects were seen among ever-smokers. Conclusions: In this cohort of middle-aged to older white men, {beta}2-AR polymorphisms were associated with airways hyperresponsiveness, particularly among lifelong nonsmokers. Our results illustrate an instance in which greater power is obtained by performing haplotype analyses as opposed to single SNP analysis.


http://www.chestjournal.org/cgi/content/abstract/126/2/509

Study objectives: Severe acute respiratory syndrome (SARS) is a rapidly progressive disease caused by a novel coronavirus (CoV) infection. However, the disease presentation is nonspecific. The aim of this study was to define clearly the presentation, clinical progression, and laboratory data in a group of patients who had SARS. Design: Retrospective observational study. Setting: A tertiary care medical center with 51 negative-pressure isolation rooms in Taipei, Taiwan. Patients: Fifty-three patients with SARS seen between April 27 and June 16, 2003. Results: Fever (ie, temperature > 38(degrees)C) was the most common symptom (98%) and the earliest. When admitted to the isolation unit of the hospital for observation, most patients reported nonspecific symptoms associated with their fever. Only two patients with preexisting illnesses had cough on the same day the fever began. Eventually, 39 patients (74%) developed cough, beginning at a mean (SD) time of 4.5 (1.9) days after fever onset, and 35 patients (66%) had diarrhea beginning at a mean time of 6.0 (3.3) days after fever onset. Thirty-one patients (59%) had abnormal findings on chest radiographs on hospital admission, and all but 1 patient (98%) eventually developed lung infiltrates that were consistent with pneumonia. The majority of
patients (63%) first developed unifocal infiltrates at a mean time of 4.5 \(\pm\) 2.1 days after fever onset, while in 37% of patients the initial infiltrates were multifocal, appearing at a mean time of 5.8 \(\pm\) 1.3 days after fever onset. Common laboratory findings included lymphopenia (on hospital admission, 70%; during hospitalization, 95%), thrombocytopenia (on hospital admission, 28%; during hospitalization, 40%), elevated lactate dehydrogenase (on hospital admission, 58%; during hospitalization, 88%), creatine kinase (on hospital admission, 18%; during hospitalization, 32%), and aspartate aminotransferase or alanine aminotransferase levels (on hospital admission, 27%; during hospitalization, 62%). Throat or nasopharyngeal swab for SARS-CoV by reverse transcriptase polymerase chain reaction (PCR) and real-time PCR was positive in 40 of the 47 patients (85%) in whom the test was performed. Conclusions: None of the presenting symptoms or laboratory findings are pathognomonic for SARS. Even though cough developed in a majority of patients, it did not occur until later in the disease course, suggesting that a cough preceding or concurrent with the onset of fever is less likely to indicate SARS. While PCR for SARS-CoV appears to be the best early diagnostic test currently available, it is clear that better methods are needed to differentiate between SARS and non-SARS illness on initial presentation.


http://www.chestjournal.org/cgi/content/abstract/127/2/430

Objectives: The recurrence of disease after the complete resection of early stage non-small cell lung cancer (NSCLC) indicates that undetected metastases were present at the time of surgery. Quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) is a highly sensitive technique for detecting rare gene transcripts that may indicate the presence of cancer cells, and endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) is a minimally invasive technique for the nonoperative sampling of mediastinal lymph nodes. The aim of this study was to determine whether these two techniques could enhance the preoperative detection of occult metastases. Methods: Patients with NSCLC were evaluated with chest CT and positron emission tomography scans. Those patients without evidence of metastases (87 patients) underwent EUS-guided FNA. Lymph nodes from levels 2, 4, 5, 7, 8, and 9 were sampled and evaluated by standard cytopathology and real-time RT-PCR. Normal control FNA specimens were obtained from patients without cancer who were undergoing EUS for benign disease (17 control specimens). For each sample, messenger RNA was extracted and real-time RT-PCR was used to quantitate the expression of six lung cancer-associated genes (ie, CEA, CK19, KS1/4, lunx, muc1, and PDEF) relative to the expression of an internal control gene (\((\beta)2\)-microglobulin). Results: Clinical thresholds of marker positivity were set at 100% specificity, as determined by the receiver operating characteristic curve analysis. Of the cytology-positive lymph nodes (27 lymph nodes), the expression of the KS1/4 gene was above its respective clinical threshold in 25 of 27 samples (93%), making this the most sensitive marker for the detection of metastatic NSCLC. At least one of the six lung cancer-associated genes was overexpressed in 18 of 61 cytology-negative patients (30%), of which KS1/4 was overexpressed in 15 of 61 patients (25%). Conclusions: Based on the high accuracy of EUS-guided FNA/RT-PCR, we predict that some of the patients in the cytology-negative/marker-positive category will have high NSCLC recurrence rates. Among the genes used in our marker panel, KS1/4 appears particularly useful for the detection of overt or occult metastatic disease.

http://circres.ahajournals.org/cgi/content/abstract/90/9/951

We studied a Syrian family with 3 children who had low-density lipoprotein cholesterol (LDL) concentrations of 13.3, 12.2, and 8.6 mmol/L, respectively. Three other siblings and the parents all had LDL values <4.52 mmol/L, suggesting an autosomal-recessive mode of inheritance. The extended pedigree had 66 additional persons with normal LDL values. A genome-wide scan in the core family with 427 markers showed support for linkage on both chromosomes 1 and 13. Markers on chromosome 1 revealed a 3.07 multipoint LOD score between 1p36.1-p35, an 18-cM interval. Surprisingly, we also found linkage to 13q22-q32, a 14-cM interval, with a 3.08 LOD score. We had identified this locus earlier as containing a gene strongly influencing LDL in another Arab family with autosomal-dominant familial hypercholesterolemia and in normal dizygotic twins. We found evidence for an interaction between these loci. We next genotyped our twin panel and confirmed linkage of the 1p36.1-p35 locus to LDL (P<0.002) in this normal population. Elucidation of ARH, the LDL receptor adaptor protein at chromosome 1p35, caused us to sequence that gene. We first identified the genomic structure of ARH gene and then sequenced the gene in our family. We found an intron 1 acceptor splice-site mutation. This mutation was not found in any other family members, in 31 nonrelated Syrian persons, or in 30 Germans. Our results underscore the importance of ARH on chromosome 1 and the chromosome 13q locus to LDL, not only in families with unusual illnesses, but also to the general population.


http://circres.ahajournals.org/cgi/content/abstract/92/3/293

Heat shock protein 72 (HSP72) is a stress-inducible protein capable of protecting a variety of cells from toxins, thermal stress, and ischemic injury. The cytoprotective role and mechanism of action of HSP72 in renal cell ischemic injury remain unclear. To study this, HSP72 was introduced (liposomal transfer) or induced (thermal stress, 43(degrees)Cx1 hour) in renal tubular cells (LLC-PK1) with Western blot confirmation. Cells were subjected to simulated ischemia 24 hours after liposomal HSP72 transfer or thermal stress, and the effect of HSP72 on nuclear factor-(kappa)B (NF-(kappa)B) activation (electrophoretic mobility shift assay and immunohistochemistry), (kappa)B (alpha) production (Western blot), postsischemic tumor necrosis factor-(alpha) (TNF-(alpha)) production (RT-PCR), and apoptosis (TUNEL assay) were determined. In separate experiments, the role of TNF-(alpha) in apoptosis was determined (anti-TNF-(alpha) neutralizing antibody). Results demonstrated that both liposomal transfer of HSP72 and thermal induction of HSP72 prevented NF-(kappa)B activation and translocation, TNF-(alpha) gene transcription, and subsequent ischemia-induced renal tubular cell apoptosis. Furthermore, TNF-(alpha) neutralization also inhibited ischemia-induced renal tubular cell apoptosis. These results indicate that liposomal delivery of HSP72 inhibits ischemia-induced renal tubular cell apoptosis by preventing NF-(kappa)B activation and subsequent TNF-(alpha) production. Further elucidation of the mechanisms of HSP-induced cytoprotection may result in therapeutic strategies that limit or prevent ischemia-induced renal damage.
Thrombomodulin (TM), a key component of the anticoagulant protein C pathway, is a major contributor to vascular thromboresistance. We previously found that TM protein expression is dramatically reduced in autologous vein grafts during the first two weeks after implantation, coincident to a local inflammatory response, and remains suppressed for at least 6 weeks. To determine the proximate cause of TM loss, in vivo gene expression was quantified by real-time PCR. TM gene expression in vein grafts declined >85% during the first postoperative week and remained suppressed >55% at 6 weeks, accounting for the observed changes in protein expression. The effects of vein graft inflammation were evaluated in animals rendered leukopenic with vinblastine before graft implantation. Abrogating the local inflammatory response affected neither TM protein nor gene expression. To determine how hemodynamic forces might modulate TM expression, the surgical protocol was modified to alter blood flow and pressure-induced vessel distension. TM protein and gene expression did not correlate to changes in shear stress but highly correlated to changes in wall tension, both acutely and over time. We conclude that the primary stimulus for altered TM expression in vein grafts is the exposure to arterial pressure. Furthermore, these data identify strain as a novel and important pathway for in vivo TM gene regulation.


http://circres.ahajournals.org/cgi/content/abstract/94/11/1515

Subject-- Peroxisome proliferator-activated receptor (PPAR)-γ agonists are emerging as potential protectors against inflammatory cardiovascular diseases including atherosclerosis and diabetic complications. However, their molecular mechanism of action within vasculature remains unclear. We report here that PPAR(γ) agonists, thiazolidinedione class drugs (TZDs), or 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) were capable of activating diacylglycerol (DAG) kinase (DGK), resulting in attenuation of DAG levels and inhibition of protein kinase C (PKC) activation. The PPAR(γ) agonist-induced DGK was completely blocked by a dominant-negative mutant of PPAR(γ), indicating an essential receptor-dependent action. Importantly, the suppression of DAG-PKC signaling pathway was functional linkage to the anti-inflammatory properties of PPAR(γ) agonists in endothelial cells (EC), characterized by the inhibition of proinflammatory adhesion molecule expression and adherence of monocytes to the activated EC induced by high glucose. These findings thus demonstrate a novel molecular action of PPAR(γ) agonists to suppress the DAG-PKC signaling pathway via upregulation of an endogenous attenuator, DGK.

Circulation (7)

Involvement of Sterol Regulatory Element Binding Protein-2-Dependent Mechanism." Circulation 106(24): 3104-3110.

http://circ.ahajournals.org/cgi/content/abstract/106/24/3104

Background-- Low-density lipoprotein (LDL) receptor-related protein (LRP) is highly expressed in vascular smooth muscle cells (VSMCs) of both normal and atherosclerotic lesions. However, little is known about LRP regulation in the vascular wall. Methods and Results-- We analyzed the regulation of LRP expression in vitro in human VSMCs cultured with native LDL (nLDL) or aggregated LDL (agLDL) by semiquantitative reverse transcriptase-polymerase chain reaction, real-time polymerase chain reaction, and Western blot and in vivo during diet-induced hypercholesterolemia by in situ hybridization. LRP expression in human VSMCs is increased by nLDL and agLDL in a time- and dose-dependent manner. Maximal induction of LRP mRNA expression was observed after 24 hours of exposure to LDL. However, agLDL induced higher LRP mRNA expression (3.0-fold) than nLDL (1.76-fold). LRP mRNA upregulation was associated with an increase on LRP protein expression with the greatest induction by agLDL. VSMC-LRP upregulation induced by nLDL or agLDL was reduced by an inhibitor of sterol regulatory element binding protein (SREBP) catabolism (N-acetyl-leucyl-leucyl-norleucinal). In situ hybridization analysis indicates that there is a higher VSMC-LRP expression in hypercholesterolemic than in normocholesterolemic pig aortas. Conclusions-- These results indicate that LRP expression in VSMCs is upregulated by intravascular and systemic LDL.


http://circ.ahajournals.org/cgi/content/abstract/105/20/2423

Background-- Reactive oxygen species, such as superoxide (O2-), are involved in the abnormal growth of various cell types. Angiotensin II (Ang II) is one of the most potent inducers of oxidative stress in the vasculature. The molecular events involved in Ang II-induced proliferation of vascular smooth muscle cells (VSMCs) are only partially understood. Methods and Results-- Ang II as well as xanthine/xanthine oxidase (X/XO) led to enhanced DNA synthesis and proliferation of VSMCs. The effect of Ang II was abolished by diphenylene iodonium. Consequently, VSMCs were incubated with X/XO, and modulation of gene expression was monitored by differential display, leading to the identification of a novel redox-sensitive gene, the dominant-negative helix-loop-helix protein Id3, which was upregulated within 30 minutes by X/XO and Ang II. Superoxide dismutase but not catalase inhibited this effect. Overexpression of antisense Id3 via transfection in VSMCs completely abolished Ang II- and X/XO-induced cell proliferation. Ang II, X/XO, and overexpression of sense Id3 downregulated protein expression of p21WAF1/Cip1, p27Kip1, and p53. Overexpression of antisense Id3 abrogated the effect of Ang II on the expression of p21WAF1/Cip1, p27Kip1, and p53. Ang II and overexpression of sense Id3 caused hyperphosphorylation of the retinoblastoma protein. Ang II-induced phosphorylation of the retinoblastoma protein was decreased by overexpression of antisense Id3. Conclusions-- Ang II induces proliferation of VSMCs via production of superoxide, which enhances the expression of Id3. Id3 governs the downstream mitogenic processing via depression of p21WAF1/Cip1, p27Kip1, and p53. These findings reveal a novel redox-sensitive pathway involved in growth control.

Background-- The delivery of autologous cells to increase angiogenesis is emerging as a treatment option for patients with cardiovascular disease but may be limited by the accessibility of sufficient cell numbers. The beneficial effects of delivered cells appear to be related to their pluripotency and ability to secrete growth factors. We examined nonadipocyte stromal cells from human subcutaneous fat as a novel source of therapeutic cells. Methods and Results-- Adipose stromal cells (ASCs) were isolated from human subcutaneous adipose tissue and characterized by flow cytometry. ASCs secreted 1203{+/-}254 pg of vascular endothelial growth factor (VEGF) per 106 cells, 12 280{+/-}2944 pg of hepatocyte growth factor per 106 cells, and 1247{+/-}346 pg of transforming growth factor-β per 106 cells. When ASCs were cultured in hypoxic conditions, VEGF secretion increased 5-fold to 5980{+/-}1066 pg/106 cells (P=0.0016). The secretion of VEGF could also be augmented 200-fold by transfection of ASCs with a plasmid encoding VEGF (P<0.05). Conditioned media obtained from hypoxic ASCs significantly increased endothelial cell growth (P<0.001) and reduced endothelial cell apoptosis (P<0.05). Nude mice with ischemic hindlimbs demonstrated marked perfusion improvement when treated with human ASCs (P<0.05). Conclusions-- Our experiments delineate the angiogenic and antiapoptotic potential of easily accessible subcutaneous adipose stromal cells by demonstrating the secretion of multiple potentially synergistic proangiogenic growth factors. These findings suggest that autologous delivery of either native or transduced subcutaneous ASCs, which are regulated by hypoxia, may be a novel therapeutic option to enhance angiogenesis or achieve cardiovascular protection.


http://circ.ahajournals.org/cgi/content/abstract/111/5/633

Background-- Drugs that simultaneously decrease platelet function and inflammation may improve the treatment of cardiovascular disorders. Here, we determined whether dipyridamole and aspirin, a combination therapy used to prevent recurrent stroke, regulates gene expression in platelet-monocyte inflammatory model systems. Methods and Results-- Human platelets and monocytes were pretreated with dipyridamole, aspirin, or both inhibitors. The cells were stimulated with thrombin or activated by adhesion to collagen, and gene expression was measured in the target monocytes. Thrombin-stimulated platelets increased monocyte chemotactic protein-1 (MCP-1) expression by monocytes. Dipyridamole but not aspirin attenuated nuclear translocation of NF-(kappa)B and blocked the synthesis of MCP-1 at the transcriptional level. Dipyridamole delayed maximal synthesis of interleukin-8 but did not alter cyclooxygenase-2 accumulation. Adherence to collagen and platelets also increased the expression of matrix metalloproteinase-9 (MMP-9) in monocytes, a response that was inhibited by dipyridamole. In this case, however, dipyridamole did not block transcription or distribution of MMP-9 mRNA to actively translating polysomes, indicating that it regulates the expression of MMP-9 protein at a postinitiation stage of translation. Dipyridamole also blocked MCP-1 and MMP-9 generated by lipopolysaccharide-treated monocytes, indicating that at least part of its inhibitory action is unrelated to its antiplatelet properties. Conclusions-- These results indicate that dipyridamole has selective antiinflammatory properties that may contribute to its actions in the secondary prevention of stroke.


http://circ.ahajournals.org/cgi/content/abstract/110/24/3727
Background-- Genetic factors have an important role in the pathogenesis of intracranial aneurysm (IA). The results of previous studies have suggested several loci. Methods and Results-- From 29 IA families with \[ \geq 3 \] individuals affected by IA, we used nonparametric (model-free) methods for linkage analyses, using GENEHUNTER and Merlin software. Genome-wide linkage analyses revealed 3 regions on chromosomes 17cen (maximum nonparametric logarithm of the odds score \[ \text{MNS} = 3.00, \text{nominal } P=0.001 \]), 19q13 (MNS=2.15, nominal P=0.020), and Xp22 (MNS=2.16, nominal P=0.019). We tested 4 candidate genes in these regions: the microfibril-associated protein 4 gene (MFAP4) and the promoter polymorphism of the inducible nitric oxide synthase gene (NOS2A) on chromosome 17cen, the epsilon genotypes of the apolipoprotein E gene (APOE) on chromosome 19q13, and the angiotensin I converting enzyme 2 gene (ACE2) on chromosome Xp22. Associations of their polymorphisms with IA were evaluated by a case-control study (100 cases: 29 probands from IA families and 71 unrelated subjects with IAs, 100 unrelated control subjects [unaffected members with IAs and absence of family history of IAs]). However, the case-control study showed that none of the polymorphisms of the examined genes had associations with IA. Conclusions-- A genome-wide scan in 29 Japanese families with a high degree of familial clustering revealed 1 suggestive linkage region on chromosome 17cen and 2 potentially interesting regions on chromosomes 19q13 and Xp22. These regions were consistent with previous findings in various populations.


http://circ.ahajournals.org/cgi/content/abstract/108/14/1724

Background-- Matrix metalloproteinase (MMP)-2 and MMP-9 have been shown to play a role in the progression of hemorrhagic stroke. We hypothesized that donor intracerebral hemorrhage (ICH) is associated with activation of the metalloproteinases before transplantation that play a key role in the subsequent development of transplant vasculopathy. Methods and Results-- We evaluated mRNA expressions of MMP-2 and MMP-9 in donor spleen lymphocytes (before transplantation) and in heart biopsies at 1 week after transplantation in 20 recipients from ICH donors and 20 recipients from trauma donors. Patients underwent serial coronary intravascular ultrasound, and interstitial myocardial fibrosis was quantified at 1 year. The baseline characteristics were similar except for increased donor age in the ICH group. Heart biopsies from the ICH group showed significant increased expression of MMP-2 (17-fold, \( P<0.0001 \)) and MMP-9 (20-fold, \( P<0.0001 \)) compared with the trauma group. Furthermore, the ICH group showed 1.8-fold (\( P=0.016 \)) increased mRNA expression of MMP-2 and 1.7-fold (\( P=0.015 \)) increased mRNA expression of MMP-9 in the donor spleen lymphocytes, suggesting the presence of systemic activation of metalloproteinases before transplantation. At 1 year, the ICH group showed increased myocardial fibrosis and accelerated coronary vasculopathy. Using multivariate regression analysis, MMP-9 was found to be associated with increased risk for vasculopathy independent of donor age (OR, 2.41; \( P=0.01; \) 95% CI, 1.24 to 4.69). Conclusions-- This is the first report to describe systemic activation of MMP-2 and MMP-9 in donors with intracerebral hemorrhage and subsequent development of allograft vasculopathy.


http://circ.ahajournals.org/cgi/content/abstract/109/10/1230

Background-- CD4+CD28null T cells are present in increased numbers in the peripheral blood of
patients with acute coronary syndrome (ACS) compared with patients with chronic stable angina (CSA). The triggers of activation and expansion of these cells to date remain unclear. Methods and Results-- Twenty-one patients with ACS and 12 CSA patients with angiographically confirmed coronary artery disease and 9 healthy volunteers were investigated. Peripheral blood leukocytes were stimulated with human cytomegalovirus (HCMV), Chlamydia pneumoniae, human heat-shock protein 60 (hHSP60), or oxidized LDL (ox-LDL). CD4+CD28null cells were separated by flow cytometry and assessed for antigen recognition using upregulation of interferon-γ and perforin mRNA transcription as criteria for activation. CD4+CD28null cells from 12 of 21 patients with ACS reacted with hHSP60. No response was detected to HCMV, C pneumoniae, or ox-LDL. Incubation of the cells with anti-MHC class II and anti-CD4 antibodies but not anti-class I antibodies blocked antigen presentation, confirming recognition of the hHSP60 to be via the MHC class II pathway. Patients with CSA had low numbers of CD4+CD28null cells. These cells were nonreactive to any of the antigens used. Circulating CD4+CD28null cells were present in 5 of the 9 healthy controls. None reacted with hHSP60. Conclusions-- We have shown that hHSP60 is an antigen recognized by CD4+CD28null T cells of ACS patients. Endothelial cells express hHSP60 either constitutively or under stress conditions. Circulating hHSP60-specific CD4+CD28null cells may, along other inflammatory mechanisms, contribute to vascular damage in these patients.

Cladistics  (1)


http://cdli.asm.org/cgi/content/abstract/11/3/625

Toll-like receptor 2 (TLR2) is critical in the immune response to mycobacteria. Herein, we report that the frequency of a human TLR2 Arg677Trp polymorphism (C2029T nucleotide substitution) in tuberculosis patients in Tunisia is significantly higher than in healthy controls (P < 0.0001). This finding suggests that this polymorphism could be a risk factor for tuberculosis.

Two PCR protocols targeting the 18S rRNA gene of Cryptococcus neoformans were established, compared, and evaluated in murine cryptococcal meningitis. One protocol was designed as a nested PCR to be performed in conventional block thermal cyclers. The other protocol was designed as a quantitative single-round PCR adapted to LightCycler technology. One hundred brain homogenates and dilutions originating from 20 ICR mice treated with different azoles were examined. A fungal burden of 3 x 10^1 to 2.9 x 10^4 CFU per mg of brain tissue was determined by quantitative culture. Specific PCR products were amplified by the conventional and the LightCycler methods in 86 and 87 samples, respectively, with products identified by DNA sequencing and real-time fluorescence detection. An analytical sensitivity of 1 CFU of C. neoformans per mg of brain tissue and less than 10 CFU per volume used for extraction was observed for both PCR protocols, while homogenates of 70 organs from mice infected with other fungi were PCR negative. Specificity testing was performed with genomic DNA from 31 hymenomycetous fungal species and from the ustilaginomycetous yeast Malassezia furfur, which are phylogenetically related to C. neoformans. Twenty-four strains, including species of human skin flora like M. furfur and Trichosporon spp., were PCR negative. Amplification was observed with Cryptococcus amyloleus, Filobasidiella depauperata, Cryptococcus laurentii, and five species unrelated to clinical specimens. LightCycler PCR products from F. depauperata and Trichosporon faecale could be clearly discriminated by melting curve analysis. The sensitive and specific nested PCR assay as well as the rapid and quantitative LightCycler PCR assay might be useful for the diagnosis and monitoring of human cryptococcal infections.


A powerful, cost-effective new method for studying single-nucleotide polymorphisms (SNPs) is described. This method is based on the use of hairpin-shaped primers (HP), which give a sensitive and specific PCR amplification of each specific allele, without the use of costly fluorophore-labeled probes and any post-PCR manipulation. The amplification is monitored in real-time using SYBR Green I dye and takes only 2 h to yield results. The HP assay has a simple design and utilizes a conventional real-time PCR apparatus. The -44 C[G] transversion in the DEFB1 gene (which encodes human (beta)-defensin 1) has been previously associated with Candida carriage in oral epithelia. In this study, we analyzed the association between early-onset periodontal disease (EOP) and the -44 SNP. We used an HP assay to study the distribution of the -44 SNP in 264 human DNAs obtained from two cohorts of EOP patients and healthy controls from different ethnic backgrounds. The results indicate that the -44 SNP has a similar distribution between EOP and healthy patients, suggesting that it is not associated with the disease.

CD14 (sCD14) released into the culture supernatants of peripheral blood lymphocytes (PBMC) from human immunodeficiency virus (HIV)-infected individuals. Monocytes from HIV-positive individuals exhibited both enhanced mCD14 expression and sCD14 production in the PBMC culture supernatants compared to the levels of mCD14 and sCD14 in HIV-negative individuals. This enhanced mCD14 expression and sCD14 production in HIV-infected individuals may be due to the effects of cytokines, the bacterial product lipopolysaccharide (LPS), and/or the HIV regulatory antigens Tat and Nef. Interleukin-10 (IL-10), an immunoregulatory cytokine, as well as LPS enhanced mCD14 expression and the release of sCD14 in the culture supernatants. HIV-Nef, unlike Tat, enhanced mCD14 expression on monocytes but did not induce the release of sCD14 into the culture supernatants. Studies conducted to investigate the mechanism underlying HIV-Nef-induced mCD14 expression revealed that HIV-Nef upregulated mCD14 expression via a mechanism that does not involve endogenously produced IL-10. In contrast, LPS upregulated the expression of mCD14 and increased the release of sCD14 via a mechanism that involves, at least in part, endogenously produced IL-10. Furthermore, dexamethasone, an anti-inflammatory and immunosuppressive agent, inhibited HIV-Nef-induced CD14 expression in an IL-10-independent manner. In contrast, dexamethasone inhibited IL-10-dependent LPS-induced CD14 expression by interfering with IL-10-induced signals but not by blocking IL-10 production. These results suggest that HIV-Nef and IL-10 constitute biologically important modulators of CD14 expression which may influence immunobiological responses to bacterial infections in HIV disease.


http://cdli.asm.org/cgi/content/abstract/11/3/538

Recurrent respiratory papillomatosis (RRP) is a chronic, debilitating disease of the upper airway caused by human papillomavirus type 6 (HPV-6) or HPV-11. We describe responses of peripheral blood mononuclear cells (PBMC) and T cells from RRP patients and controls to the HPV-11 early proteins E6 and E7. PBMC were exposed in vitro to purified E6 or E7 proteins or transduced with fusion proteins containing the first 11 amino acids of the human immunodeficiency virus type 1 protein tat fused to E6 or E7 (tat-E6/tat-E7). TH1-like (interleukin-2 [IL-2], gamma interferon [IFN-{gamma}], IL-12, and IL-18), and TH2-like (IL-4 and IL-10) cytokine mRNAs were identified by reverse transcription-PCR, and IFN-{gamma} and IL-10 cytokine-producing cells were identified by enzyme-linked immunospot assay. These studies show that HPV-11 E6 skews IL-10-IFN-{gamma} expression by patients with RRP toward greater expression of IL-10 than of IFN-{gamma}. In addition, there is a general cytokine hyporesponsiveness to E6 that is more prominent for TH1-like cytokine expression by patients with severe disease. Patients showed persistent IL-10 cytokine expression by the nonadherent fraction of PBMC when challenged with E6 and tat-E6, and, in contrast to controls, both T cells and non-T cells from patients expressed IL-10. However, E7/tat-E7 cytokine responses in patients with RRP were similar to those of the controls. In contrast, E6 inhibited IL-2 and IL-18 mRNA expression that would further contribute to a cytokine microenvironment unfavorable to HPV-specific, T-cell responses that should control persistent HPV infection. In summary, E6 is the dominant inducer of cytokine expression in RRP, and it induces a skewed expression of IL-10 compared to the expression of IFN-{gamma}.


http://cdli.asm.org/cgi/content/abstract/12/4/477
Rationale: evaluation of the T-cell receptor (TCR) V{beta}-chain repertoire by PCR-based CDR3 length analysis allows fine resolution of the usage of the TCR V{beta} repertoire and is a sensitive tool to monitor changes in the T-cell compartment. A multiplex PCR method employing 24 labeled upstream V{beta} primers instead of the conventionally labeled downstream C{beta} primer is described. Method: RNA was isolated from purified CD4 and CD8 T-cell subsets from umbilical cord blood and clinical samples using TRI reagent followed by reverse transcription using a C{beta} primer and an Omniscript RT kit. The 24 V{beta} primers were multiplexed based on compatibility and product sizes into seven reactions. cDNA was amplified using 24 V{beta} primers (labeled with tetrachloro-6-carboxyfluorescein, 6-carboxyfluorescein, and hexachloro-6-carboxyfluorescein), an unlabeled C{beta} primer, and Taqgold polymerase. The fluorescent PCR products were resolved on an automated DNA sequencer and analyzed using the Genotyper 2.1 software. Results: V{beta} spectratypes of excellent resolution were obtained with RNA amounts of 250 ng using the labeled V{beta} primers. The resolution was superior to that obtained with the labeled C{beta} primer assay. Also the numbers of PCRs were reduced to 7 from the 12 required in the C{beta} labeling method, and the sample processing time was reduced by half. Conclusion: The method described for T-cell receptor V{beta}-chain repertoire analysis eliminates tedious dilutions and results in superior resolution with small amounts of RNA. The fast throughput makes this method suitable for automation and offers the feasibility to perform TCR V{beta} repertoire analyses in clinical trials.

http://cdli.asm.org/cgi/content/abstract/10/2/195

Deficiencies of the early components of the classical complement pathway impair the actions of innate and humoral immunity and may lead to increased susceptibility to infections. We have studied the genetic basis of total C4B deficiency in a Finnish patient with recurrent meningitis, chronic fistulas and abscesses. The maternal chromosome carried a four-gene deletion including the C4B gene, and a conversion from C4B to C4A gene was found on the paternal chromosome resulting in complete deficiency of C4B. In the converted C4A gene, mutation screening did not reveal any amino acid changes or prominent mutations, yet a large number of nucleotide variations were found. Further, the patient was heterozygous for structural deficiency of mannann binding lectin (MBL) associating with medium levels of serum MBL. Our data provides new information on the genetic instability of the C4 gene region, and on the association of homozygous C4B deficiency and variant MBL genotype with increased susceptibility to recurrent and chronic infections. Importantly, plasma therapy induced a prompt clinical cure with long-term effects.

http://cdli.asm.org/cgi/content/abstract/10/1/53

Perturbations in the T-cell receptor (TCR) V{beta} repertoire were assessed in the CD4 and CD8 T lymphocytes of human immunodeficiency virus (HIV)-infected children who were receiving therapy during the chronic phase of infection by flow cytometry (FC) and PCR analysis. By FC, representation of 21 TCR V{beta} subfamilies was assessed for an increased or decreased percentage in CD4 and CD8 T cells, and by PCR, 22 TCR V{beta} subfamilies of CD4 and CD8 T cells were analyzed by CDR3 spectratyping for perturbations and reduction in the number of peaks, loss of Gaussian distribution, or clonal dominance. The majority of the TCR V{beta}
subfamilies were examined by both methods and assessed for deviation from the norm by comparison with cord blood samples. The CD8-T-lymphocyte population exhibited more perturbations than the CD4 subset, and clonal dominance was present exclusively in CD8 T cells. Of the 55 total CD8-TCR Vβ families classified with clonal dominance by CDR3 spectratyping, only 18 of these exhibited increased expression by FC. Patients with high numbers of CD8-TCR Vβ families with decreased percentages had reduced percentages of total CD4 T cells. Increases in the number of CD4-TCR Vβ families with increased percentages showed a positive correlation with skewing. Overall, changes from normal were often discordant between the two methods. This study suggests that the assessment of HIV-induced alterations in TCR Vβ families at cellular and molecular levels yields different information and that our understanding of the immune response to HIV is still evolving.


http://cdli.asm.org/cgi/content/abstract/9/1/138

We have applied a newly developed real-time reverse transcriptase (RT) PCR (RT-PCR) assay for quantification of substance P (SP) mRNA expression (the SP real-time RT-PCR assay) in human blood monocyte-derived macrophages, peripheral blood lymphocytes, and microglia isolated from fetal brain. The SP real-time RT-PCR assay had a sensitivity of 60 mRNA copies, with a dynamic range of detection between 60 and 600,000 copies of the SP gene transcript per reaction mixture. The coefficient of variation of the threshold cycle number between the SP real-time RT-PCR assays was less than 1.16%. This assay with an SP-specific primer pair efficiently recognizes all four isoforms of preprotachykinin A (the SP precursor) gene transcripts. In order to use this assay to measure the levels of SP mRNA in the human immune cells quantitatively, we designed a specific probe (molecular beacon) derived from exon 3 of the SP gene. We demonstrated that the real-time RT-PCR quantitatively detected SP mRNA in the human immune cells, among which the microglia isolated from fetal brain had the highest levels of SP mRNA. The SP real-time PCR assay yielded reproducible data, as the intra-assay variation was less than 1%. Thus, it is feasible to apply the real-time RT-PCR assay for quantification of SP mRNA levels in human immune cells, as well as in other nonneuronal cells. Since SP is a major modulator of neuroimmunoregulation, this assay has the potential for widespread application for basic and clinical investigations.


http://cdli.asm.org/cgi/content/abstract/12/4/537

The substance P (SP)-preferring receptor, neurokinin-1 receptor (NK-1R), has an important role in inflammation, immune regulation, and viral infection. We applied a newly developed real-time reverse transcription (RT)-PCR assay to quantify NK-1R mRNA in human neuronal cell line (NT-2N), a human B-cell line (IM9), monocyte-derived macrophages (MDM), peripheral blood lymphocytes (PBL), and human astroglialomas (U87 MG). The NK-1R real-time RT-PCR assay has a sensitivity of 100 mRNA copies, with a dynamic range of detection between 102 and 107 copies of NK-1R gene transcripts per reaction. This assay is highly reproducible, with an intraassay coefficient variation of threshold cycle (Ct) of less than 1.9%. The NK-1R real-time RT-PCR is highly sensitive for quantitative determination of NK-1R mRNA in human immune cells (MDM and PBL) that express low levels of NK-1R mRNA. In addition, the assay has the ability to accurately quantitate the dynamic changes in NK-1R mRNA expression in interleukin-1{beta}-stimulated U87 MG. These data indicate that the NK-1R real-time RT-PCR has potential for a
wide application in investigation of NK-1R expression at the mRNA level under physiological and pathological conditions in both the central nervous system and the immune system.


http://cdli.asm.org/cgi/content/abstract/10/6/1123

CCR5, a {beta}-chemokine receptor, plays an important role in human immunodeficiency virus (HIV) infection of human immune cells, as it is a primary coreceptor for HIV entry into macrophages. We have applied a newly developed real-time reverse transcriptase PCR (RT-PCR) assay for the quantification of CCR5 mRNA in human blood immune cells. The CCR5 real-time RT-PCR assay has a sensitivity of 100 mRNA copies, with a dynamic range of detection between 102 and 106 copies of the CCR5 mRNA transcripts per reaction. The assay is highly reproducible, with an intra-assay coefficient of variation of the threshold cycle of less than 2.07%. When used for quantification of CCR5 mRNA in human monocyte-derived macrophages (MDM) and peripheral blood lymphocytes (PBL), the assay has precision and reproducibility. MDM expressed higher levels of CCR5 mRNA than did PBL. Thus, this assay has the potential and a wide application for the investigation of the role of CCR5 in inflammatory diseases and viral infections, including HIV disease.


http://cdli.asm.org/cgi/content/abstract/9/6/1270

Rhodococcus equi infects and causes pneumonia in foals between 2 and 4 months of age but does not induce disease in immunocompetent adults, which are immune and remain clinically normal upon challenge. Understanding the protective response against R. equi in adult horses is important in the development of vaccine strategies, since those mechanisms likely reflect the protective phenotype that an effective vaccine would generate in the foal. Twelve adult horses were challenged with virulent R. equi and shown to be protected against clinical disease. Stimulation of cells obtained from bronchoalveolar lavage fluid with either R. equi or the vaccine candidate protein VapA resulted in significant proliferation and a significant increase in the level of gamma interferon (IFN-{gamma}) expression by day 7 postchallenge. The levels of interleukin-4 expression were also increased at day 7 postchallenge; however, this increase was not antigen specific. Anamnestic increases in the levels of binding to R. equi and VapA of all immunoglobulin G (IgG) antibody isotypes [IgGa, IgGb, IgG(T)] examined were detected postchallenge. The levels of R. equi- and VapA-specific IgGa and IgGb antibodies, the IgG isotypes that preferentially opsonize and fix complement in horses, were dramatically enhanced postchallenge. The antigen-specific proliferation of bronchoalveolar lavage fluid cells, the levels of IFN-{gamma} expression by these cells, and the anamnestic increases in the levels of opsonizing IgG isotypes are consistent with stimulation of a memory response in immune adult horses and represent correlates for vaccine development in foals.

To investigate a putative link between genetically determined variations in Toll-like receptor 2 (TLR2) and the occurrence of severe Staphylococcus aureus infection, the functional Arg753Gln single-nucleotide polymorphism and the GT repeat microsatellite in the TLR2 gene were examined in a large case-control study. No associations with disease or mortality attributable to these features were found.


The time course of cell-mediated and humoral immune responses was elucidated in eight women with human papillomavirus type 16 (HPV-16) infection by performing serial HPV-16 E6 and E7 cytotoxic T-lymphocyte (CTL) assays and HPV-16 virus-like particle (VLP) antibody analyses. Four subjects had a single incident of HPV-16 DNA detection, and four subjects had two periods of HPV-16 DNA detection. In two of the women in the latter group, the second episode of HPV-16 detection occurred in the presence of high titers of HPV-16 VLP antibody, bringing into question the protective role of humoral immunity in preventing repeated infection. However, all four subjects rapidly became HPV-16 DNA negative following the second detection of HPV-16 DNA, suggesting the presence of immunological memory. In addition, one subject rapidly became negative for HPV-16 DNA despite having no evidence of CTL or VLP antibody response prior to the second HPV-16 DNA detection, suggesting the presence of immunological responses at an undetectable level. Overall, seven of eight subjects (88%) had detectable HPV-16 E6 and/or E7 CTL responses and seven of eight women (88%) had detectable HPV-16 VLP antibody responses.


There is considerable controversy concerning the evidence for the presence of Chlamydia pneumoniae in the cerebrospinal fluid (CSF) of both multiple sclerosis (MS) patients and patients with other neurological diseases (OND). In order to clarify this issue, the laboratories at Vanderbilt University Medical Center (VUMC) and the University of South Florida (USF) examined the reproducibility of their respective PCR assays for the detection of C. pneumoniae DNA in the CSF of a common group of MS patients and OND controls. The two laboratories used different DNA extraction and PCR techniques in order to determine the prevalence of the C. pneumoniae genome in both monosymptomatic and clinically definite MS patients as well as in OND controls. In clinically definite MS patients, the VUMC and USF detection rates were 72 and 61%, respectively, and in patients with monosymptomatic MS, the VUMC and USF detection rates were 41 and 54%, respectively. The PCR signal was positive for 7% of the OND controls at VUMC and for 16% at USF. These studies confirm our previous reports concerning the high prevalence of C. pneumoniae in the CSF of MS patients. The presence of C. pneumoniae in patients with monosymptomatic MS would also suggest that infection with the organism occurs early in the course of the disease.

http://cdli.asm.org/cgi/content/abstract/11/2/392

The prevalence of infection with hepatitis A virus (HAV), HBV, HCV, HDV, and HEV was evaluated in 249 apparently healthy individuals, including 122 inhabitants in Ulaanbaatar, the capital city of Mongolia, and 127 age- and sex-matched members of nomadic tribes who lived around the capital city. Overall, hepatitis B surface antigen (HBsAg) was detected in 24 subjects (10%), of whom 22 (92%) had detectable HBV DNA. Surprisingly, HDV RNA was detectable in 20 (83%) of the 24 HBsAg-positive subjects. HCV-associated antibodies were detected in 41 (16%) and HCV RNA was detected in 36 (14%) subjects, none of whom was coinfected with HBV, indicating that HBV/HCV carriers account for one-fourth of this population. Antibodies to HAV and HEV were detected in 249 (100%) and 28 (11%) subjects, respectively. Of 22 HBV DNA-positive subjects, genotype D was detected in 21 subjects and genotype F was detected in 1 subject. All 20 HDV isolates recovered from HDV RNA-positive subjects segregated into genotype I, but these differed by 2.1 to 11.4% from each other in the 522- to 526-nucleotide sequence. Of 36 HCV RNA-positive samples, 35 (97%) were genotype 1b and 1 was genotype 2a. Reflecting an extremely high prevalence of hepatitis virus infections, there were no appreciable differences in the prevalence of hepatitis virus markers between the two studied populations with distinct living place and lifestyle. A nationwide epidemiological survey of hepatitis viruses should be conducted in an effort to prevent de novo infection with hepatitis viruses in Mongolia.

Clinica Chimica Acta (21)


http://www.sciencedirect.com/science/article/B6T57-3X10S21-4/2/dd9c9049fbe29d71eebe349252d43c45

Three different mutations at codons 330 (TTA to ATA), 365 (GGA to AGA) and 515 (CGT to TGT) of human butyrylcholinesterase (hBChE) were identified in a Japanese family. We correlated alterations in in the patient's hBChE activity with possible structural alterations in the three-dimensional structure of hBChE caused by the point mutations. This study was performed using the published computer-generated three-dimensional structure of hBChE based on the structure of acetylcholinesterase. The amino acid substitution at L330I was adjacent to hydrophobic residues that form the channel domain of the active center. This side chain faced the side opposite the active center. The amino acid substitution at G365R was located at the position most remote from the active center, and this substitution site was exposed to the surface of the BChE protein. [alpha]-Helical structure was present to the active center, and the guanidyl residue of native Arg 515 was hydrogen-bonded to the carboxyl group of Asp 395 in the [alpha]-helix. These point mutations may cause steric effects on the present patient's hBChE activity. This is the first report of three-dimensional structural analysis performed on the L330I, G365R, and R515C mutations of hBChE.
Background: The renin-angiotensin system (RAS) and endothelial nitric oxide (NO) affect the pathogenesis of atherosclerosis and prognosis of coronary artery disease (CAD). Previous epidemiologic data suggested that genetic factors are more likely to affect young rather than old people. Our objective was to investigate the association between the polymorphisms of eNOS (Glu298Asp) and the RAS genes and premature CAD in a Turkish population.

Methods: A total of 115 Turkish patients with premature CAD and 83 controls were included in the study. ACE I/D, AT1R A/C, AGT T/M and eNOS Glu298Asp gene polymorphisms were analysed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: It was found that increased premature CAD risk is associated with higher frequencies of the ACE DD [OR: 2.600 (CI 95% 1.395-4.847, p=0.002)], AGT MM [OR=2.407 (CI 95% 1.267-4.573, p=0.007)] and eNOS 894TT [OR=17.000 (CI 95% 3.952-73.125, p=0.002)] genotypes. The combinations ACE DD+eNOS 894TT (p=0.002), AGT MM+eNOS 894TT (p=0.001), AT1R AA+eNOS 894TT and AT1R non-AA+eNOS 894TT (p=0.002) genotypes were significantly associated with the risk of premature CAD.

Conclusions: This study indicates a synergistic contribution of RAS genes (ACE I/D, AGT T/M, AT1R T/C) and eNOS Glu298Asp polymorphisms to the development of the premature CAD.

Background: The objective of the present study was to elucidate possible relationships between four polymorphisms of the TGF-[beta]1 gene (-800G>A; -509C>T; Leu10Pro; Arg25Pro) and stage, histological activity grade and progression rate of liver fibrosis, classified according to the METAVIR-score. Methods: Three study groups, i.e. 48 patients with hepatic fibrosis (26 with known duration of hepatitis C virus infection), 47 patients with non-fibrotic diseases and 50 healthy blood donors, were analyzed for TGF-[beta]1 polymorphisms using ARMS-PCR and sequence analysis. The concentrations of total TGF-[beta]1 in plasma and of hyaluronan, P-III-NP and activities of transaminases in serum were measured. Results: The presence of proline at codons 10 and/or 25 was associated with a faster progression of fibrosis than other polymorphisms. Patients with the genotype 25ArgPro developed fibrosis significantly faster (0.23 units/year) than those having 25ArgArg (0.08 units/year). Similarly, the fibrosis progression rate of patients with genotypes 10LeuPro and 10ProPro was almost three times as fast as of those having genotype 10LeuLeu. Stage and histological activity grade of fibrosis in 25ArgPro in comparison to 25ArgArg were higher. Also 10LeuPro showed a higher average stage of fibrosis than 10LeuLeu. The TGF-[beta]1 plasma concentrations of patients with hepatic fibrosis were not significantly different between carriers of 25ArgArg and 25ArgPro genotypes. The frequency of the genotype 25ArgPro in liver fibrotic patients was about three times that of the control group whereas the frequency distribution of the genotype 25ArgArg tended to lower frequency in the fibrosis group. TGF-[beta]1-promoter polymorphisms did not show any correlation with stage, grade or progression of liver fibrosis. Conclusion: Our results indicate that the heterozygous ArgPro of codon 25 predicts significantly faster fibrotic progression of chronic hepatitis C than the
homozygous 25ArgArg genotype. The homozygous LeuLeu genotype of codon 10 showed a slow progression of fibrosis.


http://www.sciencedirect.com/science/article/B6T57-44FD01N-12/2/ec1fd57252c7c6cdb43aed356fb9ac97

Background: The prothrombin mutation, a G/A transition at position 20210 in the 3' untranslated region of the prothrombin gene, is associated with an increased risk of deep venous thrombosis and obstetrical complications. Several methods have been developed to detect the mutation; however, given the increased demand for this test in risk factor assessment, the development of simple and efficient screening methods has become necessary. Methods: We have used a rapid, sensitive, and precise method developed by Abbott Laboratories to detect the prothrombin mutation. The method employs a polymerase chain reaction (PCR) amplification and the Abbott LCx(R) microparticle enzyme immunoassay (MEIA) for detection. This method is able to detect and identify both homozygous and heterozygous genotypes. Results: Two hundred ninety-six patients with a history of deep venous thrombosis, pulmonary embolism, preeclampsia, or cardiovascular disease and 163 control patients were included in this study. The prevalence of the mutation was 5.74% in the high-risk group and 3.06% in the control group. There was complete agreement between the results from the MEIA detection with those obtained using other detection methodologies, namely standard PCR and restriction fragment length polymorphism (RFLP) analysis. Conclusions: The MEIA detection method of the prothrombin mutation represents a simple, fast, and reliable alternative to standard methods of detection and is well suited for use in routine clinical laboratories. The results of our study confirm others' studies showing a greater incidence of G20210A prothrombin gene mutation in patients with an increased risk of venous thrombosis and pulmonary embolism as well as patients with cardiovascular disease and pregnant women with preeclampsia. It reinforces the necessity of including the screening for prothrombin mutation in populations at risk.


http://www.sciencedirect.com/science/article/B6T57-47P8YNR-9X/2/a20fc7c206e283b5e91b489cccf54d6a

The discovery of a point-mutation, adenine-to-guanine, at position 985 in the gene coding for MCAD (G985), gave the basis for an easy and specific polymerase chain reaction test. We tested the specificity of such a PCR based assay and detected correctly G985 and A985 in sequence verified cDNA clones. We showed that the G985 mutation is present in genomic DNA from 48 of 50 patients with confirmed MCAD deficiency, originating from various European countries, Australia and the USA. On the basis of this high frequency of the G985 mutation among patients, we improved and optimized the assay with respect to reliability and convenience for routine diagnostic and screening purposes. As little as 2 [mu]l blood from filter-paper blood-spots (Guthrie spots) is sufficient for the test.

Background: The purpose of this study was to examine the relationship between the production of secreted fibrinogen and the synthesis of [gamma]-chain mRNA. Methods: We transfected a [gamma]-chain expression vector into Chinese hamster ovary cells already expressing both A[alpha]- and B[beta]-chains of fibrinogen and measured fibrinogen output concentrations by ELISA. We quantified both [gamma]-chain and B[beta]-chain mRNA concentrations using the recently developed TaqMan fluorogenic detection system. Results: The concentration of secreted fibrinogen into the media positively correlated with the amount of fibrinogen contained in the cell lysates. Additionally, quantitative mRNA assays revealed that the fibrinogen concentration in the cell lysates correlated well with the concentration of [gamma]-chain mRNA (r=0.7077, pr=0.0224, NS). Conclusions: These results demonstrate that the amount of recombinant fibrinogen produced in cells transfected with the [gamma]-chain vector, also expressing normal A[alpha]- and B[beta]-chains, is dependent on the transcription of [gamma]-chain mRNA. Namely, in this recombinant expression system using a two-step transfection procedure, [gamma]-chain synthesis is the rate-limiting factor for fibrinogen production. This quantitative method to measure mRNA may prove very useful for further in vivo analysis of fibrinogen gene transcription.


Background: Mutations in the erythroid-specific 5-aminolevulinate-synthase gene (ALAS2) have been identified in many cases of X-linked sideroblastic anemia (XLSA). Methods: A polymerase chain reaction-mediated restriction fragment length polymorphism (RFLP) assay was used. Results: A G527T point mutation was identified. This resulted in a substitution of tyrosine for asparagine at residue 159 (D159Y). This mutation was also identified in the mother of the two probands. Mutations in all three individuals were confirmed by DNA sequencing analysis. Conclusions: We identified a missense mutation in exon 5 of the ALAS2 gene in two brothers of a consanguineous marriage, who were clinically pyridoxine-responsive.


Background: trans-Resveratrol, or 3,5,4'trihydroxy-trans-stilbene, is a polyphenolic compound that seems to provide a protective effect against several types of cancer, notably breast cancer. Through its phytoestrogenic properties it regulates the expression of hormone-dependent genes, such as the oncosuppressor BRCA1, in breast cells. This gene is involved in the majority of hereditary breast cancer, as well as sporadic cancers. Methods: We used three human breast tumor cell lines (HBL100, MCF7 and MBA-MB-231) and one breast cell line (MCF10a) derived from a fibrocystic disease to study in vitro the effect of resveratrol on the transcription of a group
of genes whose proteins interact in different pathways with BRCA1. BRCA1, BRCA2, ER [alpha], ER [beta], p53, p21waf1/cip1, CBP/P300, RAD51, pS2 and Ki67 mRNA were quantified using real-time quantitative RT-PCR with an ABI 7700 apparatus. Results: Resveratrol modulated the expression of these genes in a pattern dependent on the status of [alpha] and [beta] estrogen receptors. These results show that resveratrol regulates gene expression via the estrogen receptor pathway and also an undetermined pathway. Conclusion: Thus, resveratrol seems to have an effect on breast tumor cell lines, on a fibrocystic cell line by affecting several factors regulating the function of BRCA1.


http://www.sciencedirect.com/science/article/B6T57-44YF86G-1/2/11b4db70f6e2168c51eb15c4636f

Background: We examined a technique for detecting point mutations of K-ras codon 12 in stool samples using one-step polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis, in order to determine whether it could be used to screen for colorectal cancer. Methods: DNA was extracted from 200-mg stool specimens of 5 healthy controls and 31 colorectal cancer patients. A 107-base-pair fragment of exon 1 of K-ras was amplified by PCR using mismatched primers. PCR products were digested with Bst NI and analyzed by gel electrophoresis followed by silver staining. Specificity of one-step PCR/RFLP was examined by using synthetic oligonucleotides. The detection limit of K-ras codon 12 mutations was determined by using SW480 and HT29 cells. Results: The K-ras gene was successfully amplified from all healthy controls and colorectal cancer patients studied. Mutations of K-ras codon 12 were not detected in any of the healthy controls, but were identified in 13 (41.9%) of the 31 patients with colorectal cancer. Mutations were detectable in all six synthetic mutant DNAs, while none were detected among the wild type. The detection limit of this method was >=0.1%. Conclusions: PCR/RFLP analysis could be used in mass screening for colorectal cancer, because it is highly specific, has a low detection limit, and is simpler than conventional methods for detecting genetic abnormalities.


http://www.sciencedirect.com/science/article/B6T57-4DRBFHP-2/2/7fa321e7b52f4c9c4ae6b14b3d9b80b3

Background: Butyrylcholinesterase (BCHE) deficiency is characterized by prolonged apnea after the use of certain muscle relaxants with the genetic defect lying in the BCHE gene. Methods: Two Chinese patients with no serum BCHE activity were studied. The BCHE genes were screened for mutations by polymerase chain reaction and direct DNA sequencing. Results: Of the four mutations detected, two novel mutations were identified in the two patients, i.e., F474L, and an insertion of an adenine between nucleotide positions 395 and 396. This information was used to screen the immediate families of the patients for carrier status. Conclusions: We established the molecular basis of butyrylcholinesterase deficiency in two Chinese patients. The developed mutation detection assay provides a reliable method for identifying mutant BCHE carriers.

http://www.sciencedirect.com/science/article/B6T57-43N683V-F/2/7e150fd0af824c9464d8ca99d8063cd9


http://www.sciencedirect.com/science/article/B6T57-45DFDRN-2/2/97969a34af6b662bc54ba1652f4da74b

Background: The tumour necrosis factor-\[alpha\] (TNF[alpha]) promoter polymorphism (-308 G/A) has been shown to be associated with the susceptibility to and/or the severity of diverse diseases such as infections, autoimmunity, and malignancies. We developed a genotyping technique based on the mutagenically separated polymerase chain reaction (MS-PCR) which may be useful in the clinical risk assessment. Methods: Different length allele-specific primers and an unspecific complementary strand primer were used in a one-tube assay. At least one PCR product was generated in a single reaction obviating the need for an internal control amplification. Introduction of additional base substitutions into the allele-specific primers led to a clear-cut separation between the alleles through the reduction of cross-reactions during amplification. The only post-PCR step required was the separation of allelic PCR products by size upon agarose gel electrophoresis. Results: The allele frequencies in 300 German healthy Caucasians were 0.84 for TNF1 (-308 G) and 0.16 for TNF2 (-308 A) in accordance with published data obtained with the conventional RFLP method. No significant deviation from Hardy-Weinberg equilibrium was observed. The specificity of MS-PCR was confirmed by sequence-based typing. Conclusions: MS-PCR is a rapid, reliable, and cost-effective technique for genotyping of the TNF[alpha] promoter polymorphism (-308 G/A).


http://www.sciencedirect.com/science/article/B6T57-4384MXN-4/2/4dee516690124c757bbc635338dfac5a

Cytochrome P450 (CYP450) mixed-function mono-oxygenases, consisting of more than 30 enzymes, are responsible for the metabolism of a large number of drugs and metabolites. With the rapid advances in the human genome project, the role of genetic polymorphism in drug metabolism may become an important adjunct for rational drug therapy, and for the explanation of drug toxicity and interactions. This preliminary study modified a previously described procedure for genotyping CYP2D6*3 and *4. An additional step included uracil-DNA glycosylase for the prevention of "carry-over" contamination. DNA was extracted from peripheral blood using PureGene DNA Isolation kit. CYP2D6*3 and *4 sequences were amplified by PCR, followed by digestion with restriction endonuclease Msp1 and Mva1, respectively. Resulting fragments were analyzed by electrophoresis and visualized by ethidium bromide staining. Poor metabolizers of *3 mutation showed 168-, 82- and 20-bp bands, while those of *4 showed a single 355-bp band. Using these protocols, 22 individuals were genotyped, showing the following prevalence for *3 and *4: 0 and 3, respectively--comparable to those of the general population. This method provides a reliable means of genotyping CYP2D6*3 and *4.
We quantitatively measured the amount of recombinant molecules formed during PCR when the break point cluster region (BCR) cDNA was co-amplified with a homologous internal standard using Taq polymerase. The products were analysed under denaturing conditions using capillary electrophoresis followed by detection of the fluorescently labelled products and the recombinant molecules were differentiated by their size. Early termination of chain synthesis and reannealing of incomplete fragments, to each other as well as to BCR and internal standard, is one mechanism for generating recombinants during PCR since prolonging extension time reduced, but did not totally suppress recombinant molecule formation. Template switching by the extending chain is another mechanism since recombinant molecules could be detected even after only one round of primer extension. The latter mechanism is probably facilitated by increasing number of templates. Thus, the large increase of recombinant molecules formed in plateau phase is mediated by direct amplification of the recombinants and de novo synthesis by template switching. The effect of additives on recombination could be quantitatively measured and both betaine and DMSO were effective in suppressing recombination. Thus, prolonging extension time, reducing the number of amplification cycles and incorporating additives in the PCR reaction, reduced recombinant molecule formation.

The prevalence of a mutation of the codon for tryptophan 64 to arginine (Trp64Arg) in the [beta]3-adrenergic receptor gene was investigated by genotyping 261 Japanese subjects. The allelic frequency of this mutation was 0.18. Subjects with the homozygous W64R mutant alleles had a significantly higher prevalence of fatty liver, BMI, serum [gamma]-glutamyl transpeptidase, and serum leucine amino transpeptidase levels than those without the mutation. Individuals with this mutation also showed a higher fasting blood glucose level than those without this mutation. However, the prevalence of diabetes mellitus was no different between the three groups. These results suggest a potential association of the Trp64Arg mutation with higher morbidity of fatty liver and mild glucose intolerance.
Heterologous expression of human plasma procarboxypeptidase U (proCPU, TAFI) was obtained in mammalian cells (C127 and DON) and in insect cells (Sf21 and H5 cells). Conditioned media were purified by cation-exchange chromatography and plasminogen affinity chromatography to yield an essentially pure protein. Results: All systems gave high expression levels (6-20 mg/l). Due to differences in glycosylation of the activation peptide, the recombinant variants of proCPU migrated differently on SDS-PAGE (52-65 kDa). However, after activation, all active recombinant enzymes migrated at 35 kDa, similar to native CPU and no evidence for post-translational modification of the catalytic domains could be detected. For the mammalian cell produced variants, activation was more efficient after desialylation. After activation, CPU showed low solubility (0.2 mg/ml) but was inhibited similarly as native CPU. Conclusions: Mammalian cell systems were the most efficient for the production of human plasma recombinant proCPU. The obtained zymogen differs with respect to the extent and the heterogeneity of glycosylation but, after activation, the experiments did not reveal any alteration between the recombinant and native protein.


http://www.sciencedirect.com/science/article/B6T57-40R5B2S-4/2/9d60d036eb2e054b73e007af6acd2430

We describe an immuno-polymerase chain reaction (immuno-PCR) assay for the detection of human angiotensinogen using identical first and second polyclonal antibodies. The reporter DNA was initially generated by PCR amplification using a biotinylated primer, and was bound with streptavidin to biotinylated second antibody. Human recombinant angiotensinogen sandwiched by antibodies was detected by amplifying the reporter DNA using PCR. To reduce the effect of nonspecific amplification, the optimal concentrations of streptavidin and DNA label were determined to be 0.1 mg/l and 0.5 ng/l, respectively. The detection limit of the immuno-PCR assay was 0.1 ng/l, an approximately 2.5 x 105-fold improvement compared with a conventional enzyme-linked immunosorbent assay. These results indicate that a highly sensitive immuno-PCR for human angiotensinogen can be developed even with identical first and second polyclonal antibodies.


http://www.sciencedirect.com/science/article/B6T57-3SNJTP-2/2/8f8b0c80a5ca0e59b53ec6992ba53fa9

The aims of this study were two-fold: first, to assess the relative diagnostic performance of non-isotopic in situ hybridization (ISH) and the nested polymerase chain reaction (nested-PCR) applied to Epstein-Barr virus (EBV) detection in a series of 55 unselected nasopharyngeal carcinoma (NPC) cases and, secondly, to correlate these data with histopathological classification. Our study shows that in 76.36% of NPC cases positive nuclear signals were observed using EBV-ISH. Overall, EBV-ISH positivity varied according to histological type, in that undifferentiated carcinomas showed a higher proportion of positive cases than differentiated cell carcinomas, although ISH results do not show significant differences in relation to histological types when employing two different schemes (WHO and Micheau). However, in adequate quality DNA samples (54 NPC cases), EBV-DNA was detected in 100% of cases using a nested-PCR, supporting the previous view that all histological types of NPC are in reality variants of EBV-
infected neoplasia. ISH-negative cases probably reflect a lower sensitivity than PCR, particularly when a small number of viral copies are present, as well as a variable technical effectiveness for detected EBV, independent of the NPC histological type.


http://www.sciencedirect.com/science/article/B6T57-45J9464-3/2/26cc768298e9044c9d24c96dd2f1e212

Background: BRCA1 and BRCA2 are breast cancer susceptibility genes. Recent studies suggest that BRCA1 interacts with a great variety of proteins, including BRCA2, cell-cycle regulators, transcriptional activators and repressors. We investigated the expression of both BRCA1 and BRCA2 during the progression of the cell cycle of human tumor cell lines from different origins (MCF7, MDA-MB231, PA1 and CCL221) in two growth status (60% and 100% of confluency).

Methods: First, the growth status was characterized by determination of the cell cycle by flow cytometry analysis. At the same time, immunohistochemistry was performed to follow BRCA1 and BRCA2 protein expression and then, quantification of BRCA1 and BRCA2 transcripts was realized using real-time quantitative RT-PCR. Results: We reported in studied tumor cell lines with 60% of confluency by comparison with 100% of confluency, an increase in the BRCA1 and BRCA2 expression at the level of proteins and transcripts. Conclusion: Therefore, the expression of both BRCA1 and BRCA2 genes at the protein and mRNA levels appear to be up-regulated after cell proliferation in human tumor cell lines from different origins.


http://www.sciencedirect.com/science/article/B6T57-3Y4BP2Y-1/2/6e724b1fc63f1f66b663193aa01a0e5bf

Telomerase is an enzyme that synthesizes and adds repetitive telomeric sequences of (TTAGGG)n to the ends of chromosomes. Recently, several telomerase-associated genes have been cloned, making it possible to study the expression of these genes. Quantitative comparisons of the expression of these genes and of telomerase activity might help clarify the regulation of telomerase activity. Therefore, we established the validity of a quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay for the human telomerase catalytic subunit (hTERT) mRNA and telomerase associated protein (TEP1) mRNA using the TaqMan(TM) fluorogenic detection system. Using this assay, we quantitated hTERT mRNA and TEP1 mRNA expression in two human pancreatic cancer cell lines, AsPC-1 and PANC-1. Our results indicated that the levels of hTERT mRNA and TEP1 mRNA expression in AsPC-1 were 1.50 and 2.31 times higher than in PANC-1 cells. This TaqMan(TM) RT-PCR assay appears to be useful in determining the quantities of hTERT and TEP1 mRNAs in clinical specimens. Taken together, our results indicate that it is possible to measure the expression of the major telomerase genes subunits. Furthermore it is possible to apply this technique to determine the amount of other types of mRNA.

[beta]-2 adrenergic receptor (B2AR) agonists are the most widely prescribed rescue agents used in the treatment of asthma. Recent studies have indicated a relationship between a polymorphism at codon 16 of the B2AR gene, and the response to recurrent [beta]-agonist therapy. The B2AR polymorphism of interest involves a single nucleotide change from A to G, resulting in an amino acid change from Arginine (Arg) to Glycine (Gly). Clinical efforts to further investigate this relationship require an accurate, reliable and inexpensive method for detecting the polymorphism. In this study, we report an LCx(R) assay for the detection of a single nucleotide polymorphism at codon 16 of the [beta]-2 adrenergic receptor. This assay is capable of detecting patients harboring any of the three possible genotypes at this locus, namely, homozygous wild type, homozygous variant or heterozygous individuals with a single genomic DNA sample of 25-500 ng. It requires minimum hands-on time with automated detection. The assay would be suitable for use in research labs for screening of a large number of samples. We believe that this type of assay will facilitate research and clinical investigations in elucidating the association of SNPs with disease states, diagnosis, prognosis and treatment.

Clinical and Diagnostic Virology (6)


Background: The presumed latency of cytomegalovirus (CMV) in leucocytes and the sensitivity of the polymerase chain reaction (PCR) raise a question of its clinical value. Objectives: To develop and standardize a CMV PCR as a diagnostic tool for CMV infection in solid organ and bone marrow transplant patients by comparing it to a likewise standardized isolation, rapid isolation and to clinical symptoms. Study design: The material comprised 822 EDTA peripheral blood samples from 96 solid organ and 119 bone marrow transplant patients. One sample from each of 21 healthy bone marrow donors and 25 blood donors were used as controls. Two million leucocytes were lysed and one-tenth of a volume was used in a nested PCR employing immediate early gene primers. Results: The limit of detection was [ap] 10 gene copies of a CMV DNA clone and 1 TCID50 of extracted DNA from a cell suspension. The specificity was >=0.99 when tested in CMV seronegative individuals. The positive and negative predictive values were 0.62 and 1.00, respectively. When PCR was compared to virus isolation/rapid culture in individual patients, PCR was positive more frequently in solid organ transplant patients than was CMV isolation/rapid culture, but the difference was not significant in bone marrow transplant patients. In isolation-positive patients, PCR became positive in samples taken 1-2 weeks earlier. In 54 solid organ transplant patients with PCR-positive samples, CMV-associated symptoms were present in 29/31 patients with CMV isolated from blood but in only 5/23 patients without viraemia. In 17 bone marrow transplant patients treated with ganciclovir, PCR became negative during or immediately after treatment in 14/20 (70%) episodes. This was true of 5/12 (42%) solid organ transplant patients. Conclusion: Screening of transplant patients with CMV PCR can be standardized at a clinically relevant level so that antiviral therapy can be instituted early.
Background: Multiplex polymerase chain reaction (PCR) has been established as a general technique for the simultaneous amplification of different target sequences. Uses of multiplex include pathogens identification, linkage analysis and genetic disease diagnosis. The high sensitivity of PCR may produce false-positive results due to contamination with previously amplified material. Objectives: To develop a multiplex PCR technique that can simultaneously detect and discriminate human immunodeficiency virus types 1 and 2 (HIV-1/2) and human T-lymphotropic virus types 1 and 2 (HTLV-I/II) proviral sequences. Such a method should incorporate a system that prevents the occurrence of false-positive results. Study design: Combinations of four primer pairs, one for each retrovirus, were assayed in order to determine the combination of oligonucleotides as well as the PCR conditions that yield the most specific and sensitive coamplification of proviral sequences. To prevent contamination with DNA from previous PCR amplifications, the uracil N-glycosylase (UNG) system was incorporated into the coamplification format. Results: A combination of primer pairs from the gag region of HIV-1, env of HIV-2, pol of HTLV-I and tax of HTLV-II yielded specific and sensitive coamplification of proviral sequences. The UNG system was incorporated and shown to be efficient in the degradation of contaminating DNA. In the evaluation of a serologically well established panel of singly and dually infected individuals, the assay detected 20/22 HIV-1, 8/10 HIV-2, 8/8 HTLV-I and 8/8 HTLV-II infections. Conclusions: A multiplex PCR method for the detection and discrimination of HIV-1/2 and HTLV-I/II has been developed. Under standardized conditions, all four proviral sequences were detected in a specific and sensitive manner. The evaluation of a panel of clinical specimens from infected individuals by one or more retroviruses showed that the technique detected most of the infected individuals. A low viral load may explain cases where multiplex PCR failed to detect target sequences.

http://www.sciencedirect.com/science/article/B6T58-3W7BDK-B/2/02152d1a99b2c226c6f65468e69c49dfc

Background: Insulin-dependent diabetes mellitus or type 1 diabetes is a disease with a diverse aetiology. Epidemiological studies examining newly diagnosed, recent onset IDDM patients have suggested a role for viruses in the aetiology of IDDM (Yoon, 1995, Diabetes/Metabolism Reviews 11, 83-107). Important candidates are the enteroviruses, in particular coxsackieviruses B3 and B4. The latter can cause diabetes in animals (Clements et al., 1995, Lancet 346, 221-223). Objectives: We have developed a quantitative PCR method for the detection of enterovirus genomes in biological samples. The quantitative PCR will be used to screen for enteroviruses in blood of diabetes patients and their relatives by testing a Blood Diabetes Register. Study design: A substantial amount of data has been collected on enterovirus induced IDDM, our study is original in so far as it will be: (1) a quantitative study, not only the presence of viral genome sequences in blood will be determined, but also their concentrations (viral load); and (2) a longitudinal study, samples are and will be collected as a function of time. Positive PCR samples will be quantified using the standard addition method. Results: The test is specific for enteroviruses, since all enteroviruses were detected with equal sensitivity. Viruses belonging to other picornavirus genera scored negative (even up to 3 x 10^6 genome copies). An equal detection limit of 10 genome copies was found for all enteroviruses. Conclusions: The developed method will permit us to generate quantitative and longitudinal data of enterovirus genomes in blood of diabetes patients and their relatives, which might help in the elucidation of the relationship between enteroviruses and IDDM.


http://www.sciencedirect.com/science/article/B6T58-3W3FH73-5/2/9a052a75d6c7f2609d02d6a0d3e5bb3

Background: Rapid laboratory methods for the early detection of cytomegalovirus (CMV) are needed for the prevention of CMV disease in transplant recipients. These methods should not only be able to detect the virus but also be highly predictive for CMV disease. Objective: The clinical value of a simple and rapid nested plasma polymerase chain reaction (PCR) was evaluated by comparing the results with CMV pp65 antigen detection in leukocytes (CMV antigenemia assay), virus isolation from leukocytes, CMV IgG and IgM antibody response and clinical data. Study design: A total of 471 EDTA blood samples were collected from 85 kidney transplant patients during a 3-4 month period after transplantation. CMV DNA was amplified directly from 10 [mu]l of plasma while 150 000 separated leukocytes were stained for CMV pp65 antigen by each of two monoclonal antibodies. A total of one million leukocytes were used for virus isolation. The PCR protocol used in the present study involves a simple alkaline lysis technique for isolating DNA directly from plasma which is easy and rapid to perform. Results: Twenty-eight patients developed symptomatic CMV infection while asymptomatic infection occurred in 29 patients. CMV pp65 antigen detection had a 75% sensitivity and a 57% positive predictive value for CMV disease development, compared with 64% and 79% sensitivity and 49% and 46% positive predictive value for CMV DNA and viremia, respectively. The median time until detection of CMV in patients with symptomatic CMV infection was 26 days after transplantation,
compared with 49 days in asymptomatic patients by any of the methods used. Early appearance (within 8 weeks) of CMV pp65 antigen and CMV DNA had high predictive values for symptomatic infection; repeated detection of pp65 antigen and CMV DNA were more common in symptomatic patients. Conclusions: CMV antigenemia assay and plasma PCR can be used for pre-symptomatic diagnosis of CMV infection. Virus isolation and CMV serology in most cases provide a post-symptomatic diagnosis. The best marker for monitoring kidney transplant patients might be the quantitative CMV antigenemia assay.


http://www.sciencedirect.com/science/article/B6T58-3YK00V6-G/2/70f3d9c60fc80619e9bfe30c883f64eb

Background: The detection of proviral DNA by Polymerase Chain Reaction (PCR) is regarded as an important tool in the diagnosis of HIV-1 infection, specially among adults at risk of AIDS and children born to seropositive mothers. However, application of PCR in routine testing is hampered by the need to use radioactive probes. Objectives: In this study, a non-radioactive test based on a microtiter plate (DNA Enzyme ImmunoAssay, DEIA) was used for the detection of proviral sequences of HIV-1 in peripheral blood cells of different patients. The results of the PCR-DEIA assay were compared to those obtained by liquid hybridization (PCR-LH), virus isolation (VI) and Western blot (WB).

Study design: The study population included 92 patients belonging to three different groups: seropositive subjects with a well-defined clinical status and WB profile; adults at risk of infection with negative or indeterminate WB; children born to seropositive mothers with still unestablished HIV-1 infection.

Results: In the seropositive subjects, both PCR-LH and PCR-DEIA confirmed infection and gave the same results as WB. In adults at risk of infection, PCR with both methods anticipated the seroconversion in one patient with indeterminate WB and confirmed the absence of infection among seronegative and other indeterminate patients. In children born to seropositive mothers, both PCR systems as well as VI permitted an early diagnosis of infection, as confirmed by the clinical follow-up.

Conclusion: This study has shown that in subjects at risk of AIDS and in children born to seropositive mothers, the non-isotopic DEIA method presents the same sensitivity and specificity for the detection of HIV-1 infection as the radioactive procedure. The DEIA method appears to be particularly useful for the detection of PCR products in routine diagnostic analyses.

Clinical Biochemistry (24)


http://www.sciencedirect.com/science/article/B6TDD-3YG4F6-65/2/af951a23a7b6f8874d6a0a458e3b4e26

Objective: We used single-strand conformational polymorphism (SSCP). To screen for mutations/polymorphisms in exon 4 of the apolipoprotein C III in 45 patients with hypertriglyceridemia and 46 control individuals, single-strand conformational polymorphism was
investigated using restriction endonuclease and amplification refractory mutations systems (ARMS). Results: SSCP identified six patterns corresponding to six genotypes. We confirmed that the different genotypes result from the two polymorphic sites at positions 3175 and 3206 of the apo C III gene. Only three of four possible haplotypes were found in the study population. This resulted in the identification of 6 of the 10 possible genotypes. Conclusions: SSCP is a useful method to screen for both known and unknown mutations/polymorphisms and should have increasing applications in clinical laboratories involved with the study of genetic markets of a wide variety of diseases.


http://www.sciencedirect.com/science/article/B6TDD-4DW3F3H-4/2/d5f9809162ed946788e651d676e3e730

Objective: We examined several critical parameters that must be optimized when converting between the ABI Prism 7700 real-time PCR platform and the Cepheid SmartCycler[trademark] II while using the same primer and probe sequences. Design and methods: A lyophilized master mix, MgCl2 concentration, PCR cycling conditions, and ramp times were evaluated. Results: Optimization of each parameter, including use of the OmniMix[trademark] HS-lyophilized beads, 6 mM MgCl2 concentration, changes in PCR cycling parameters, and increased ramp time were necessary to convert this real time PCR assay to a new platform. Conclusion: We conclude that careful consideration of several analytical parameters can result in a smooth transition of assays between real time PCR platforms.


http://www.sciencedirect.com/science/article/B6TDD-4D0Y760-1/2/75626ac41f0985d8c3de4cddf1c92bfb

Objectives: To assess the impact of the human Fc[gamma]RIIA and Fc[gamma]RIIIB gene polymorphisms on the risk of rheumatic fever (RF). Designs and methods: Fc[gamma]RIIA-R/H-131 and Fc[gamma]RIIIB-NA1/NA2 genotypes were determined using polymerase chain reaction in 66 RF cases and 117 healthy controls in this case control study. Results: Compared with healthy controls, the RR genotype was enriched in the entire group of RF cases (odds ratio [OR] 4.98, 95% confidence interval [95% CI] 1.81-13.70). RF patients were more frequently HR heterozygotes rather than HH homozygotes (OR 3.09 vs. 0.11). The results of this study show that patients who have RF are more likely to have the RR and HR genotypes than control children. These probabilities show that RR is associated with the greatest risk for rheumatic fever and HR is associated with an intermediate risk. For the distribution of Fc[gamma]RIIIB NA2 genotypes, a nonsignificant increase was found in RF patients (39.31% vs. 51.51%; OR 1.64, P = 0.1226). Conclusion: The Fc[gamma]RIIA-R/H-131 polymorphism may be an important marker in determining predisposition to RF.

Objectives: Head and neck cancer is one of the ten most frequent cancers in the world. The angiogenic growth factors VEGF, PDGF and bFGF play a role in cancer aggressiveness. We developed a sensitive method to quantify the gene expression of these factors in the tissues of head and neck cancer patients.

Design and methods: All assays were performed using real-time RT-PCR, which yields a value (Ct) denoting the threshold cycle of PCR amplification at which product is first detected by fluorescence. The Ct is dependent on the quantity of the target molecule in the sample. To control for variation in RNA quantity and quality, we used 18S ribosome RNA as an internal control to calculate a relative Ct for the target molecules of interest, VEGF, PDGF and bFGF. A serially diluted positive control sample was analyzed by linear regression to determine the sensitivity and linearity of the assay. Paired normal and cancerous tissue samples from 115 head and neck cancer patients were assayed to ascertain the relative levels of the growth factors.

Results: The CVs of within-run and between-run assays for VEGF, PDGF and bFGF were all less than 3%. The correlation coefficient of the RNA concentrations and Ct values were 0.9987, 0.9977, and 0.9996 respectively for VEGF, PDGF and bFGF. The assay was sensitive to as little as 10-3 ng of RNA. All three growth factors were significantly increased in tumor tissue as compared to normal tissue. VEGF, PDGF and bFGF levels were elevated in 71.3%, 58.2% and 54.0% of cancerous tissue samples, with average levels of over-expression of 35.1, 24.6 and 13. sixfold, respectively.

Conclusion: This method provides sensitive, quantitative, high-throughput analysis for direct comparison of gene expression levels between samples, while adjusting for factors that may influence quantity determination. It should be applicable to molecules other than angiogenic growth factors, as well.


More than 95% of the patients with chronic myelogenous leukemia (CML) carry translocations between protooncogene abl of chromosome 9 and bcr gene of chromosome 22, resulting in the Philadelphia chromosome (Ph1). After allogeneic bone marrow transplantation (BMT) it is important to detect possible residual malignant cells in CML patients. A new sensitive hybridization method combined with polymerase chain reaction (PCR), based on the detection of the europium (Eu3+) label by time-resolved fluorescence, was applied for the detection of Ph1 chromosome. Total RNA from 106 peripheral blood leukocytes was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction. After cDNA synthesis by reverse transcriptase, the PCR amplification (30 cycles) was carried out. In the detection phase two oligonucleotide probes were used in the hybridization reaction, one biotinylated (bcr gene, exon 2) and one (abl gene) labeled with Eu3+. The hybrids were collected in a streptavidin-coated microtitration well and the bound Eu3+ was measured in a time-resolved fluorometer. To assess the sensitivity of the method, different numbers of CML cell line K562 cells were mixed with 105 apparently normal human leukocytes. Five K562 cells/105 leukocytes could be detected. Six patients with CML confirmed by clinical and cytogenetic criteria were studied. Three of the patients underwent an allogeneic BTM 6-18 months before the investigation and all of them were Ph1-negative. The other three patients who were nontransplanted were positive as expected.


http://www.sciencedirect.com/science/article/B6TDD-4287BN8-6/2/0492c4a24be7f1362c9030e524c6236

Objectives: The major RNase activity of leukocytes has been attributed to eosinophil-derived neurotoxin EDN. Depletion of eosinophils enables RT-PCR from 105 leukocytes without RNA extraction. In this study we introduced streptavidin-coated PCR tube strips for the selection of eosinophil-free leukocytes for RT-PCR analysis.

Design and methods: Polypropylene 0.2 ml PCR tube strips were coated with streptavidin and biotinylated antibodies against cell surface antigens were attached to the tubes. CD7-positive T-lymphocytes, CD19-positive B-lymphocytes and CD16-positive cells (mainly neutrophils and monocytes) were positively selected by incubating of 1-2 x 105 leukocytes in the antibody-coated PCR tubes for 30 min at 23[deg]C.

Results: The mean amount of cells bound into a tube was 31,500 (CV25%) T-cells and 8,600 (CV61%) B-cells from 12 blood samples, and 23,600 (CV22%) CD16+ cells from 17 samples. The influence of selected cell lysate on the RT-PCR analysis of Philadelphia chromosome (bcr/abl translocation) from 100 K562 cells was small: 78% (CV28%) of the leukocyte-free signal was obtained in the presence of CD16+ cells or 89% (CV15%) and 99% (CV11%) and in the presence of T-cells and B-cells, respectively.

Conclusions: These results suggest that through the introduction of eosinophil-free cell population into RT-PCR a reproducible method with reasonable leukocyte yield and avoiding RNA extraction was developed.


http://www.sciencedirect.com/science/article/B6TDD-46HG2R3-3/2/d2108cab5786f9f29482c90b6fa4e7c

Objective: To develop tetra-primer PCR assays for detection of the CCR2-V64I, CCR5-A59029G and SDF1-G801A polymorphisms associated with HIV pathogenesis.

Design and Methods: For each assay, two primers for the amplification of the gene locus are combined in one tube with two primers for the subsequent allele specific amplification (ASA). In the first set of cycles, pre-amplification of the gene region of interest is ensured by the gene specific primers. In the second set of cycles, lowering the annealing temperature allows ASA on the newly produced template.

Results: Analysis of 90 DNA samples resulted in allele frequencies for CCR2-V64I, CCR5-A59029G and SDF1-G801A which are similar to other Caucasian cohorts. Furthermore, re-analysis of sequenced genomic DNA by tetra-primer PCR analysis (7-11 times) always showed identical results.

Conclusion: Our set of single-tube assays allows rapid and reproducible genotyping of the CCR2-V64I, CCR5-A59029G and SDF1-G801A polymorphisms. These inexpensive but accurate assays are valuable for screening these polymorphisms in cohorts of HIV-infected patients.


http://www.sciencedirect.com/science/article/B6TDD-3V4C29HG/2/6004aeb6a60c327a2f797f2339a35f21

Kaiser, R., P.-B. Tremblay, et al. (2002). "Validity of PCR with emphasis on variable number of tandem
Objectives: Variable number of tandem repeat polymorphisms (VNTR) are frequently analyzed by PCR in genetic, epidemiologic and forensic studies. We wanted to explore the validity of these PCR analyses.

Design and Methods: The amplification of the different alleles of the 17- and the 44-bp VNTR of the serotonin transporter gene and the 39-bp VNTR of the glycoprotein Ib[alpha] gene was analyzed. We studied the effects of the parameters magnesium, dimethylsulfoxide, 7-deaza-dGTP, formamide, betaine, PCR temperatures and different types of polymerases.

Results: In all three VNTR polymorphisms selective amplification of one of the alleles of heterozygous individuals could be obtained by change of the magnesium concentration. This problem could be minimized by a combination of Taq- and Pwo-polymerases and by use of 7-deaza-dGTP.

Conclusion: PCR analysis of all of these VNTRs may give reproducibly wrong results in truly heterozygous subjects due to selective amplification of only one of the alleles.


http://www.sciencedirect.com/science/article/B6TDD-3X1VV26-9/2/d548eee19c4e077f09592f21c52448f2


http://www.sciencedirect.com/science/article/B6TDD-453FWWW-C/2/1abe0ce93f7da5479e4f5daace32a3b5

Objective: To develop a real-time PCR technique for detection of the insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) gene.

Design and methods: Three primers were designed for performing real-time PCR in the presence of SYBR Green I as fluorochrome followed by melting curve analysis. Forty human genomic DNA that have been genotyped by two-rounds of conventional PCR were used for evaluation of this technique.

Results: Melting curve analysis indicated the melting peak at 73.9[deg]C and 76.2[deg]C corresponding to the presence of I and D alleles, respectively. Comparable genotyping results were obtained by both conventional and real-time PCR. Besides, the mistyping of ID allele individuals by the first run of conventional PCR were accurately genotyped by single-tube real time PCR.

Conclusions: The real-time PCR method presented in this study provides a rapid and sensitive way for genotyping of ACE gene that may be suitable for large-scale clinical and epidemiologic study.


http://www.sciencedirect.com/science/article/B6TDD-41MB0F4-9/2/8189242a09d84ac229e372efb884fee2

Objectives: Acute intermittent porphyria (AIP) is an autosomal dominant inherited disease caused by a decreased activity of hydroxymethylbilane synthase (HMBS). As far as the gene abnormalities of the HMBS, many different mutations have been reported. In this work, we investigated the presence of mutations in a Japanese family with AIP.

Design and Methods: A 44-year-old Japanese male and nine members of his family were investigated. All of them were screened by traditional biochemical markers. Mutational analysis was performed using polymerase chain reaction-single strand conformation polymorphism method followed by DNA sequencing. A reliable restriction enzyme cleavage assay was established for the pedigree analysis.

Results: The mutation was a splicing mutation, a C to G transversion at position -3 of the acceptor site of intron 11 of the HMBS gene, resulting in the exon 12 skipping. The patient is heterozygous for the mutation, and his father appeared to be the source of the mutant allele. This mutation created a new cleavage site of the Nla III restriction enzyme and could be screened by an amplified fragment from genomic DNA with digestion. Using this cleavage assay, an asymptomatic carrier in the family was definitively identified.

Conclusions: This mutation was first found among Japanese AIP patients, but happened to be the same as reported previously from Europe. A similarity of gene abnormality may suggest that those European and Japanese AIP families have a common ancestor. Molecular investigations on the family members should be applied not only for more accurate diagnosis, but also for understanding the molecular genetic heterogeneity underlying this dominantly inherited enzymopathy.


Objectives: In the clinical laboratory, identification of Streptococcus pneumoniae can be confused with other streptococci. Conventional biochemical tests such as optochin sensitivity and bile solubility can give inconsistent results. This report presents a method to distinguish true S. pneumoniae from other upper respiratory tract streptococci when conventional tests fail.

Design and Methods: We used arbitrarily primed polymerase chain reaction with the single primer M13 universal as a method to distinguish S. pneumoniae from other upper respiratory tract streptococci.

Results: The fingerprint pattern of S. pneumoniae was established by amplifying DNA of S. pneumoniae type strains 1-48 and of other common upper respiratory tract streptococci at three different DNA concentrations with the single primer M13 universal. From these type strains, a common arbitrarily primed-polymerase chain reaction pattern was identified characterized by two predominant bands of equal intensity at 800 base pairs and at 1100 base pairs. Fingerprint patterns of viridans streptococci were easily distinguishable from those of S. pneumoniae. Many of the clinical isolates used in this study were equivocal by conventional tests but were distinguishable by their fingerprint patterns.

Conclusions: Our results indicated that the fingerprint pattern of S. pneumoniae is species specific and distinguishes true S. pneumoniae of clinical isolates from other streptococci when conventional biochemical tests are unclear.

Familial defective apolipoprotein B-100 (FDB) is a genetic disorder resulting from a mutation in the apolipoprotein B-100 (apo B-100) gene, most frequently at position 3500, in which arginine is substituted for glutamine in the mature protein. This mutation drastically decreases the affinity of the mutant apo B-100 particle for the low-density lipoprotein (LDL) receptor, and hence decreases the clearance of cholesterol from the circulation. Familial hypercholesterolemia (FH), also a disorder of lipid metabolism, results from mutations in the gene for the LDL receptor. Both FDB and heterozygous FH occur at approximately the same frequency (1 in 500) among Caucasians and both produce clinical symptoms and signs that can be indistinguishable. Polymerase chain reaction (PCR) amplification and subsequent restriction analysis have been used to detect the substitution at codon 3500 in the apo B-100 gene using mutagenic PCR primers. At least one proband from 10 unrelated families with a history of hypercholesterolemia was screened by mutagenic PCR for FDB. Only one of 10 patients demonstrated the mutation for FDB. The mutant apo B-100 allele was shown to segregate with other clinically affected family members. These results demonstrate that molecular analysis is essential to distinguish between FDB and heterozygous FH in hypercholesterolemic families.


Objective: The aim of the present study was to examine if absence of a common allele in a microsatellite polymorphism in the insulin-like growth factor I (IGF-I) promoter was associated with type 2 diabetes and alterations in quantitative traits in glucose-tolerant subjects. Methods: The IGF-I promoter polymorphism was investigated in a case-control study comprising 694 type 2 diabetic patients and 218 glucose-tolerant control subjects, and in two genotype-quantitative trait studies involving 208 glucose-tolerant first-degree offspring of type 2 diabetic patients and 218 unrelated middle-aged subjects with normal glucose tolerance. Results: No associations were found between the lack of the common promoter allele and type 2 diabetes (P = 0.25) or estimates of glucose metabolism in glucose-tolerant subjects. Presence of the wild-type allele was associated with an increase in fasting serum triglyceride levels in the group of 208 glucose-tolerant first-degree offspring of type 2 diabetic patients (P = 0.002). This finding was replicated in an independent sample of 218 unrelated middle-aged subjects with normal glucose tolerance (P = 0.007). Conclusion: The present study provides evidence that the common wild-type allele of the IGF-I promoter polymorphism is associated with increased levels of fasting serum triglyceride in glucose-tolerant whites.
Objectives: To search for mutations in the 5'-UTR and proximal promoter region of the folate receptor-[alpha] (FR-[alpha]) gene, whose exons are known to be virtually free of genetic variation in the population.

Design and methods: Seven hundred seventy-eight patient samples were screened for mutations between nt -116 and nt +207 in the FR-[alpha] gene using single strand conformation polymorphism (SSCP) followed by DNA sequencing.

Results: Three patients were found to have a 25-bp deletion, c.109_133delCCACTAAACCACAGCTGTCCCCTGG, and three others had a 1-bp A insertion, c.-69dupA, so that 0.77% of the patient population showed genetic variation already in the 323 bp promoter sequence studied so far.

Conclusions: The promoter region of FR-[alpha] may harbor much more genetic variation than its highly conserved exons, and not just isolated, unique mutations. This could be a new factor contributing to gene-food interaction explaining part of the hyperhomocysteinemia panorama. Extended searches for polymorphisms further upstream in the FR-[alpha] gene are warranted.


Objective: The presence of small numbers of cells of donor origin in the circulation of recipients of organ transplants (microchimerism) may correlate with immunologic tolerance. As part of our ongoing studies on microchimerism, we evaluated the utility of seven PCR-based assays for the detection of the less abundant DNA in paired mixtures (100 ng total DNA).

Design and methods: DNA samples were screened to identify pairs informative for one or more PCR assays. DNA mixtures from the informative pairs were then analyzed using at least one assay. The assays were based on the X-Y homologous region; a Y chromosome microsatellite locus; three autosomal microsatellite loci; the D1S80 minisatellite locus; and sequence specific oligonucleotide probe (SSOP) analysis of the HLA DRB1 locus.

Results: About 0.1% of male DNA against a background of female DNA was detectable using primers for the X-Y homologous region, but the sensitivity was increased to 0.0001% using nested primers for the Y chromosome microsatellite marker. Analysis of the minor DNA component was difficult with the three autosomal microsatellite assays because of the presence of shadow bands. Similar problems with the D1S80 assay were resolved using more stringent PCR conditions, and the sensitivity was 0.1%. Using the DRB1 locus, we were able to detect 1% DNA in the mixed samples.

Conclusions: These studies show that: (a) nested PCR for the Y chromosome is the most sensitive assay for the detection of microchimerism; (b) D1S80 is a useful marker for microchimerism; (c) additional optimization of analytical conditions is required if autosomal microsatellite markers and the SSOP assay are to be used for microchimerism analysis.

We have developed a method to genotype variable number of tandem repeats (VNTRs) and insertion/deletion polymorphisms using an integrated microfluidic chip-based system. We used this method to analyze a) a highly polymorphic pentanucleotide repeat (CCTTT)n locus within the 5'-putative promoter region of the human inducible nitric oxide synthase gene (iNOS5) which is associated with diabetic complications and infectious diseases; b) a bi-allelic 27 bp VNTR region within intron 4 of endothelial nitric oxide gene (eNOS27) which is associated with hypertension in type 2 diabetes patients with coronary heart disease and excess risk of advanced diabetic nephropathy in type 1 diabetes patients and c) an insertion/deletion polymorphism within the gene encoding angiotensin-converting enzyme (ACE/ID) which is associated with cardiovascular pathology and nitric oxide activity, and is in strong linkage disequilibrium with functional variants. Following amplifications, samples were mixed with gel-dye and markers and loaded into commercially available microfluidic chips designed for DNA sizing applications. In the study (N = 230), 95 (41%) of the DNA samples were homozygous and 135 (59%) were heterozygous for the iNOS5 repeats. For eNOS27, 173 (75%) of the genotyped DNA samples were homozygous for the larger 4b allele and the remaining 57 samples (25%) were heterozygous (4b/4a). No DNA samples were homozygous for the shorter 4a allele with four 27 bp repeats. In case of ACE/ID, 47 (20%) of the DNA samples were homozygous for the insertion, 65 (28%) were homozygous for the deletion and the remaining 118 (51%) were heterozygous. The results obtained were verified by analyzing random amplicons using bi-directional sequencing and GeneScan(R) 3.0 analyses with 100% concordance being observed. Using the microfluidic chip-based method, separation and DNA sizing and genotyping are rapidly accomplished. The DNA fragments are resolved clearly and the system allows quantitation. Finally, the microfluidic chip-based method may be used for both large- and small-scale genotyping studies.


Objective: We used single-strand conformational polymorphism (SSCP) to screen for mutations at nucleotides 833 and 919 of the cystathionine [beta]-synthase (CBS) gene in 13 patients with homocystinuria and 11 of their relatives.Methods: Exon 8 of genomic DNA was selectively amplified by PCR using primers derived from intronic sequences of the human CBS gene. SSCP analysis was performed on the amplified products. Genotypes identified by SSCP were confirmed by DNA sequencing and an allele-specific PCR method.Results: SSCP identified 5 patterns corresponding to five genotypes. We confirmed that the different genotypes result from mutations at nucleotides 833 and 919 of the CBS gene, and that these 2 mutations account for approximately 50% of affected alleles in homocystinuria patients.Conclusion: Our recent elucidation of intron-exon borders and intronic sequences of the CBS gene has made possible the use of SSCP to screen for known/unknown mutations in the CBS gene. Because T833C and G919A represent the two most common mutations and both are located within exon 8 of the CBS gene, SSCP of exon 8 allows screening of the heterozygous carrier state of these mutations in a large population, to determine the importance of heterozygosity of CBS mutations as the cause of mild hyperhomocyst(e)inemia associated with premature vascular diseases.


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http://www.sciencedirect.com/science/article/B6TDD-41MB0F4
Objective: We developed a quantitative reverse-transcription polymerase chain reaction (RT-PCR) to determine CK20 expression in colorectal tumor and hematopoietic tissue. Design and Methods: Our method incorporates a calibrated PCR with an internal competitor and an external standard. Results: The RT-PCR assay is sensitive detecting 10 target molecules of CK20 in solution with one round of 38 amplification cycles. Genomic DNA contamination was eliminated by Dnase I digestion of total RNA. The inclusion of a calibrator in the quantitative RT-PCR analysis allowed for a high throughput of unknown samples within the same assay improving comparative analysis between the samples tested. Analysis of peripheral blood and bone marrow from 20 healthy volunteers revealed a low level of CK20 expression in all samples. Conclusion: To study the clinical significance of CK20 expression as a marker of systemic metastatic disease it is essential to measure CK20 mRNA levels in hematopoietic tissue with sensitive quantitative RT-PCR. A sensitive and reproducible method, which is easily performed, is described.


http://www.sciencedirect.com/science/article/B6TDD-41MB0F4-D/2/8d5543b1fede6e32119bfdd625cc0cc68


http://www.sciencedirect.com/science/article/B6TDD-4BDC5C4-1/2/e377f8194e288112501ae90b093e7e63

Objectives: (1) To compare two stool antigen EIAs (HpSA, FemtoLab) and PCR of ureaseA and cagA in feces, with 13C-urea breath test (UBT). (2) To ascertain whether a simplified UBT (breath collection TIME = 10 min) is as reliable as the standard assay (30 min). Design and Methods: Helicobacter pylori status was recorded in Group 1 (n = 187) by UBT, H. pylori stool antigen, ureA and cagA PCR in feces. UBT with 10, 20 and 30 min sampling was performed in Group 2 patients (n = 283). Results: The sensitivity and specificity of HpSA, FemtoLab, and ureA were 67% and 99%, 90% and 96%, 35% and 98%, respectively. cagA results were positive in 16/48 H. pylori-positive, and in 5/100 H. pylori-negative patients. The results of UBT with a 10- and 30-min sampling strictly overlapped. Conclusion: UBT with 10 min breath collection and FemtoLab stool antigen assay are the most reliable non-invasive tests to diagnose H. pylori infection.

Clinical Immunology  (2)


http://www.sciencedirect.com/science/article/B6WCJ-4FH0D8G-1/2/abcc89c737c6c969e09e6994d5dba34f
After the provision of highly active antiretroviral therapy (HAART), the level of circulating CD4+ T cells increases in many adults infected with the human immunodeficiency virus, type 1 (HIV). To study factors involved in immune reconstitution, we have measured thymic abundance by CT scans, circulating naive-phenotype CD4+ T cells by flow cytometry, and T cell receptor (TCR) rearrangement excision circles (TRECs) by quantitative PCR in 40 virologically suppressed, HIV-infected adults and 33 age-matched, HIV-uninfected controls. In HIV-uninfected subjects, naive T cell numbers, thymic abundance, and the frequency of circulating naive CD4+ T cells bearing TRECs decreased with age, as expected. When corrected for this relationship with age, naive T cell numbers correlated significantly with naive T cell TREC frequencies. Virologically suppressed HIV-infected subjects had higher TREC frequencies, and subjects over the age of 39 were more likely to have abundant thymus compared to age-matched, HIV-uninfected adults. Nevertheless, all HIV-infected subjects had reduced absolute numbers of naive T cells, irrespective of thymic size, age, or TREC frequencies. These data illustrate the complex relationship between these measures of thymic size and function and underscore the need to develop more definitive measures of thymic function in the future.


http://www.sciencedirect.com/science/article/B6WCJ-4CWRKX2-1/2/ce0f00981aafcf1d0c7b962da3bb60c4

T-cells are causally involved in the pathogenesis of inflammatory bowel disease (IBD). The tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) regulates T-cell proliferation and survival. We show in this report that IDO mRNA is markedly induced in lesional colonic biopsies of IBD patients. IDO is primarily expressed in CD123+ mononuclear cells infiltrating the submucosal areas of the inflamed lesions. In Crohn's disease (CD), IDO is also strongly expressed in perifollicular regions of lymphoid follicles. Upregulation of IDO is of functional significance, as we detected an increase of kynurenine and of the kynurenine/tryptophan ratio in supernatants from colonic explant cultures (CECs) of CD patients. Immunohistochemistry of colonic biopsies taken from CD patients prior and after treatment with the TNF-blocking antibody Infliximab revealed reduced IDO expression in patients with good clinical response to Infliximab. In summary, high local expression of IDO may represent an anti-inflammatory mechanism tempting to counterbalance the tissue-damaging effects of activated T-cells infiltrating the colonic mucosa in IBD.

Clinical Neurology and Neurosurgery (1)


http://www.sciencedirect.com/science/article/B6T5F-3RSP3FH-2/2/1fe4109af4ea69742beaed9b0600dc80

Hyperekplexia (MIM: 149400), or startle disease, is an autosomal dominant neurological disorder characterized by an extreme generalized stiffness immediately after birth, normalizing during the first years of life. Other features of this disorder are excessive startle reactions to unexpected,
particularly auditory, stimuli together with a short period of generalized stiffness during which voluntary movements are impossible. Linkage analysis mapped a gene for this disorder to chromosome 5833-q35. Subsequently, mutations in the GLRAI gene encoding the [alpha]1-subunit of the glycine receptor proved to be causally related to the disease. In the present study, mutation analysis of all exon and flanking intron sequences of this gene was performed in sporadic patients and their parents. Moreover, a branch of the original Dutch hyperekplexia family with a very severely affected individual was screened for an additional mutation in the GLRAI gene. Except for two polymorphisms, of which one results in an amino acid change, no potentially disease causing mutations were found in the [alpha]1-subunit of the glycine receptor. Together with haplotype analysis these results exclude a recessive inheritance or new mutation etiology in these hyperekplexia-like syndromes and emphasize that hyperekplexia-like syndromes can be caused by other genetic factors. The involvement of other genes encoding subunits of the functional glycine receptor complex has not been excluded.

Clinical Nutrition (1)


http://www.sciencedirect.com/science/article/B6WCM-48M7V31-5/2/ec6220d5448bf0424de753db663340fb

Objective: The aim of our study is to determine the effect of a 30-day-period caloric restriction (CR) upon the immune response of rats and the influence of glutamine upon mononuclear cells proliferation and cytokine production.

Methods: Male albino Wistar rats were submitted to CR receiving an amount of food equivalent to 50% of the mean amount consumed by the control animals. We measured the incorporation of [2-14C]-thymidine by lymphocytes obtained from the spleen and mesenteric lymph nodes, plasma glucose and glutamine concentration, as well as cytokine production by cultivated cells, in the presence of glutamine.

Results: Rats submitted to CR presented reduced body weight (49%) and decreased splenic leukocyte number. CR led to a reduction in the proliferative response of lymphocyte. Spleenocytes from CR animals produced less [gamma]-interferon and interleukins 1, 4 and 10 in 48 h culture than did those from control rats. The same pattern is observed in cells obtained from the mesenteric lymph nodes. The addition of glutamine 2 mM to the culture medium restored spleen and mesenteric lymph node cells' proliferative response and the production of interleukin 2 by cells obtained from the spleen and from the mesenteric lymph nodes.

Conclusions: The present data reinforce that undernutrition decreases in vitro immune cell function and indicates that, in such circumstances, glutamine supplementation could reverse some of the changes observed in the functionality of cultured immune cells. The presence of the amino acid at physiological concentration, however, reinforces the diversion of the immune response towards a Th1-like response.

Clinical Pharmacology & Therapeutics (14)
Background and objective: Surfactants used in pharmaceutical formulations can modulate drug absorption by multiple mechanisms including inhibition of intestinal P-glycoprotein (P-gp). Our objective was to analyze the effect of 2 surfactants with different affinity for P-gp in vitro on the intestinal absorption and bioavailability of the P-gp substrate talinolol in humans.

Methods: In vitro, the influence of surfactants on talinolol permeability was studied in Caco-2 cells. In vivo, an open-label 3-way crossover study with 9 healthy male volunteers was performed. Subjects were intubated with a 1-lumen nasogastrointestinal tube. The study solution, containing either talinolol (50 mg), talinolol and D-[alpha]-tocopheryl polyethylene glycol 1000 succinate (TPGS) (0.04%), or talinolol and Poloxamer 188 (0.8%), was administered through the tube.

Results: TPGS, but not Poloxamer 188, inhibited the P-gp-mediated talinolol transport in Caco-2 cells. In healthy volunteers TPGS increased the area under the plasma concentration-time curve with extrapolation to infinity (AUC0-\([\infty]\)) of talinolol by 39% (90% confidence interval, 1.10-1.75) and the maximum plasma concentration (Cmax) by 100% (90% confidence interval, 1.39-2.88). Poloxamer 188 did not significantly alter the AUC0-\([\infty]\) or Cmax of talinolol.

Conclusions: This in vivo intraduodenal perfusion study showed that low concentrations of TPGS, close to the concentrations that showed P-gp inhibition in vitro, significantly increased the bioavailability of talinolol. The study design excluded modulation of solubility by TPGS and unspecific surfactant-related effects. The latter was supported by the absence of modulation of the talinolol pharmacokinetics by Poloxamer 188, which does not modulate P-gp. Therefore we consider intestinal P-gp inhibition by TPGS as the major underlying mechanism for the increase in talinolol bioavailability.


http://www.sciencedirect.com/science/article/B6WCN-4F02D2W-B/2/bdd068908ec347f5d374abdd63c24c27

Purpose: Irinotecan, a drug widely used in the treatment of advanced colorectal cancers, is a prodrug requiring activation to 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterase 2 (hCE2). The existence of functional polymorphisms in the gene encoding this enzyme could explain the individual variability in drug efficacy and toxicity. We have explored this possibility in looking for single nucleotide polymorphisms and their functional consequence.

Methods: In a series of 115 human deoxyribonucleic acid samples, we have explored the 12 exons of the hCE2 gene, the intron-exon junctions, and the 5’- and 3’-untranslated regions, by denaturing HPLC and sequencing of polymerase chain reaction products. The functionality of the variations identified was studied in 60 human liver samples by measuring hCE2 gene expression by real-time reverse transcriptase-polymerase chain reaction of messenger ribonucleic acid extracts and carboxylesterase activity by use of irinotecan as a substrate.

Results: We have identified a total of 11 single nucleotide polymorphisms, none of them able to alter the amino acid sequence of the protein. They are distributed in 10 distinct genotypes in addition to the wild type. The most frequent variation (localized in IVS10) has an allele frequency of 0.17 and has been identified at the homozygous state in 1 sample. hCE2 gene expression and carboxylesterase activity in the variants identified were not significantly different from those measured in wild-type samples.

Conclusion: The hCE2 gene presents several polymorphisms, none of which seems to be involved in significant variations in protein activity and, therefore, in irinotecan activation.


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-F7/2/b185948eaf7705b6a9f262ac407e50d0

Objective: To investigate the CYP2C19 polymorphism in Tanzanians because this enzyme shows large interindividual differences in activity and metabolizes several drugs of importance in Africa, especially the antimalarial agent chloroguanide (INN, proguanil).

Methods: Two hundred fifty-one Tanzanian healthy volunteers were phenotyped with respect to CYP2C19 with use of a single oral dose of mephenytoin (n = 106), a single oral dose of omeprazole (n = 207), or both. Sixty-two were phenotyped with both probe drugs. The urinary 0- to 8-hour S/R-mephenytoin ratio and the plasma omeprazole metabolic ratio (MR) (omeprazole/hydroxyomeprazole) 3 hours after drug intake were determined. The genotype was determined by analysis for CYP2C19*1 (wt), CYP2C19*2 (m1), and CYP2C19*3 (m2). Ten subjects with high omeprazole MR were screened for new mutations in the CYP2C19 gene by searching for single-strand conformation polymorphisms (SSCP).

Results: Eight subjects were classified as mephenytoin poor metabolizers (7.5%). Only 5 of these were homozygous for mutated alleles. The S/R ratio was skewed to the right (lower CYP2C19 activity) compared with other ethnic groups studied previously. No new mutations were found with polymerase chain reaction (PCR)-SSCP. We found 30 volunteers (14.5%) with an MR >7, which is the antimode found previously in white subjects and Asian subjects. Of the 251 volunteers genotyped, 3.2% were homozygous for mutated alleles and 66.1% were homozygous for the wild-type allele. The allele frequencies of CYP2C19*1, *2, and *3 were 81.5%, 17.9%, and 0.6%, respectively. The correlation between the S/R-mephenytoin ratio and the omeprazole MR was significant (Spearman r = 0.59; P

Conclusion: Tanzanians
have a decreased capacity to metabolize both omeprazole and mephenytoin when their genotype is compared with metabolic capacity and genotype in other previously studied populations. We identified a low frequency of the Asian allele (CYP2C19*3). Although we did not find any new mutations, our results may be consistent with the presence of yet-unidentified mutations of CYP2C19 that causes decreased CYP2C19 activity in the Tanzanian population.

Ieiri, I., T. Kubota, et al. (1996). "Pharmacokinetics of omeprazole (a substrate of CYP2C19) and comparison with two mutant alleles, CYP2C19m1 in exon 5 and CYP2C19m2 in exon 4, in Japanese subjects." Clinical Pharmacology & Therapeutics 59(6): 647.

http://www.sciencedirect.com/science/article/B6WCN-4FCJG4K-34/2/b516dfddfcfe959c0a416a7bf93b55e2d

The pharmacokinetic profile of omeprazole was examined in 27 healthy Japanese volunteers, and the results were analyzed in relation to genotype for the two mutations, CYP2C19m1 in exon 5 and CYP2C19m2 in exon 4, associated with the poor metabolizer phenotype. Of the 27 individuals analyzed, 10 were homozygous for the wild-type (wt) allele in both exon 5 and exon 4 (wt/wt; 37.0%, pattern G1), five were heterozygous for the CYP2C19m1 (wt/m1; 18.5%, G2), five were heterozygous for the CYP2C19m2 (wt/m2; 18.5%, G3), three were heterozygous for the two defects (m1/m2; 7.4%, G4), and five were homozygous for the CYP2C19m1 (m1/m1; 18.5%, G5). The allele frequencies of the m1 and m2 mutation were 0.31 and 0.13, respectively. A correlation between the rate of metabolism of omeprazole and genotype was observed. The mean clearance values of omeprazole in patterns G1, G2, G3, G4, and G5 were 1369.0, 332.7, 359.0, 70.8, and 89.5 ml/hr/kg, respectively. The relative area under the serum concentration-time curve (AUC) ratio of omeprazole to 5-hydroxyomeprazole in patterns G1, G2, G3, G4, and G5 was 1:2.8:3.4:16.17.2. A similar relation was observed in the omeprazole/5-hydroxyomeprazole serum concentration ratio, determined 3 hours after drug intake (1:3:4:18.8:20.3). There were significant (p < 0.05 to 0.01) differences in the disposition kinetics of omeprazole between the subjects with patterns G1, G2, and G3 and the subjects with patterns G4 and G5. The results indicate that the 5-hydroxylation pathway of omeprazole is clearly impaired in subjects with m1/m2 and m1/m1.


http://www.sciencedirect.com/science/article/B6WCN-45SR862-38/2/12eb0943c758280e3cb606718251a7da

MDR1 (P-glycoprotein) is an important factor in the disposition of many drugs, and the involved processes often exhibit considerable interindividual variability that may be genetically determined. Single-strand conformational polymorphism analysis and direct sequencing of exonic MDR1 deoxyribonucleic acid from 37 healthy European American and 23 healthy African American subjects identified 10 single nucleotide polymorphisms (SNPs), including 6 nonsynonymous variants, occurring in various allelic combinations. Population frequencies of the 15 identified alleles varied according to racial background. Two synonymous SNPs (C1236T in exon 12 and C3435T in exon 26) and a nonsynonymous SNP (G2677T, Ala893Ser) in exon 21 were found to be linked (MDR1*2) and occurred in 62% of European Americans and 13% of African Americans. In vitro expression of MDR1 encoding Ala893 (MDR1*1) or a site-directed Ser893 mutation (MDR1*2) indicated enhanced efflux of digoxin by cells expressing the MDR1-Ser893 variant. In vivo functional relevance of this SNP was assessed with the known P-glycoprotein drug substrate fexofenadine as a probe of the transporter's activity. In humans, MDR1*1 and MDR1*2 variants were associated with differences in fexofenadine levels, consistent with the in vitro data, with the area under the plasma level-time curve being almost 40% greater in the *1/*1 genotype.
compared with the *2/*2 and the *1/*2 heterozygotes having an intermediate value, suggesting enhanced in vivo P-glycoprotein activity among subjects with the MDR1*2 allele. Thus allelic variation in MDR1 is more common than previously recognized and involves multiple SNPs whose allelic frequencies vary between populations, and some of these SNPs are associated with altered P-glycoprotein function. (Clin Pharmacol Ther 2001;70:189-99.)


http://www.sciencedirect.com/science/article/B6WCN-4FBYNB9-C/2/ecb5ae21d76a5fead169831142fa5999

Background: Ritonavir is a potent inhibitor of cytochrome P4503A4 that strongly increases saquinavir bioavailability. In this study we assessed the safety and antiretroviral efficacy of the combination of these two compounds in patients pretreated and receiving continued treatment with zidovudine and lamivudine who were protease inhibitor naive and who had a CD4 cell counts below 200/mm3. Methods: In this 48-week pilot study, all patients received 600 mg ritonavir and 400 mg saquinavir twice daily. Administration of zidovudine and lamivudine was continued without a change in previous doses. Viral load, CD4 cell count, and the emergence of resistance to the two protease inhibitors were evaluated repeatedly up to week 48. Results: Sixteen patients were included in the study. Previous nucleoside analog treatment duration was 48 +/- 22 months (mean +/- SD). Two patients quit taking both protease inhibitors within 2 weeks. The ritonavir dose had to be reduced in 10 other patients because of side effects. Between inclusion and week 48, plasma viremia varied from 4.87 +/- 0.43 to 3.00 +/- 1.29 log10 copies/mL and CD4 cell counts ranged from 98 +/- 61 to 250 +/- 139/mm3. Ten patients (63%) had viral loads below 200 copies/mL and 7 (44%) had viral loads below 50 copies/mL. A single key mutation that conferred ritonavir resistance 184V and V82A/V developed in two patients. A mutation at codon 54 developed in another patient. These mutations were associated with repeated cessations of antiretroviral treatment. No lipodystrophy was observed. Conclusion: Ritonavir and saquinavir in combination are quite well tolerated and induce a high and sustained antiretroviral efficacy. A four-drug combination that includes these two protease inhibitors should be considered as a first line of treatment in patients with low CD4 cell counts.


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-N/2/712acd37aafa5912a686336e4f5fdace

The frequency of various genotypes of arylamine N-acetyltransferase (NAT2) was investigated in 248 Polish unrelated children. Allele-specific polymerase chain reaction (PCR) was applied for mutation at 341 nucleotide (nt) of NAT2 coding sequence and PCR/restriction fragment length polymorphism for the other mutations. Genotypes coded for slow acetylation in 62.9% (56.6% to 68.9%). The frequency of specific NAT2 alleles was *4 (wild-type), 22.0%; *5A (341C, 481T), 5.2%; *5B (341C, 481T, 803G), 33.1%; *5C (341C, 803G), 6.0%; *6A (282T, 590A), 30.0%; *7B (282T, 857A), 3.4%; and *12A (803G), 0.2%. No mutations were found at 191, 434, and 845 nt. By a molecular-genetic procedure, genotypes *4/*6A were confirmed not to mask *6B/*13 (590A/282T). *6B and *13 were absent in a composite sample representative of 826 alleles (95% confidence limits, 0% to 0.45%). Five cases of genotype-phenotype discrepancy were sequenced and their mutation allocation confirmed; 21 further genotypes were confirmed by sequencing. This first evaluation of NAT2 genes among a Slavic population should provide a basis for clinical and
epidemiologic investigations of NAT2 in the Polish population.


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-D3/2/fa0bfb000936a627dcd7cf9263cdd583

Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurine drugs. TPMT activity is regulated by a common genetic polymorphism that is associated with large individual variations in thiopurine toxicity and efficacy. We previously cloned the functional gene for human TPMT and reported a common variant allele for low enzyme activity, TPMT*3A, that contains point mutations at cDNA nucleotides 460 and 719. In the present study, we set out to determine the number, types, and frequencies of TPMT variant alleles associated with low enzyme activity in clinical laboratory samples in the United States and to compare those results with data obtained from two different ethnic groups. We identified a total of six different variant alleles for low TPMT activity in the 283 clinical laboratory samples studied. The most common variant was *3A; the second most frequent variant allele, *3C, contained only the nucleotide 719 polymorphism; and four other variant alleles were detected. TPMT*3A also appeared to be the most common variant allele in a Norwegian white population sample, but it was not found in a population sample of Korean children. However, *3C was present in samples from the Korean children, as was a novel allele, *6. Characterization of variant alleles for low TPMT enzyme activity will help make it possible to assess the potential clinical utility of deoxyribonucleic acid-based diagnostic tests for determining TPMT genotype.


http://www.sciencedirect.com/science/article/B6WCN-4CNTHGM-H/2/4ce8a27f46ae515ec92f0487733a606

Aim: The flavin-containing monooxygenase 3 (FMO3) has been shown to be genetically polymorphic. In vitro, the enzyme contributes to the N-oxidation of clozapine, caffeine, and several other drugs. We therefore wanted to analyze population frequencies and allelic linkage of FMO3 mutations and their functional effect on the metabolism of clozapine and caffeine. Methods: This study included 204 patients treated with clozapine for schizophrenia and 192 healthy volunteers receiving a 100 mg oral test dose of caffeine. FMO3 polymorphisms M66I, P153L, E158K, V257M, E305X, E308G, and R492W were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Ratios of serum clozapine N-oxide over clozapine and of urine theobromine versus paraxanthine were used as in vivo indicators of FMO3 activity. Results: From the known FMO3 amino acid variants, only K158 (frequency 0.426), G308 (0.225), and M257 (0.069) were found; mutations I66, L153, X305, and W492 were not found in the 396 subjects. Linkage analysis revealed seven different alleles; the most frequent of these was the wild-type E158-V257-E308 (0.534), followed by K158-V257-G308 (0.199) and K158-V257-E308 (0.192). Subjects with these frequent variants of FMO3, however, did not differ in clozapine N-oxidation or caffeine oxidation compared with the wild-type. Conclusion: There are several genetic polymorphisms for the FMO3 enzyme. The effects on the metabolism of caffeine or clozapine could not be shown, indicating that the mutations have only minor functional effects or that substrate affinity is too low to be clinically relevant. (Clin Pharmacol Ther 1999;66:431-8.)
Objective: To determine the possible impact of CYP2D6 polymorphism on the pharmacokinetics and pharmacodynamics of selegiline.

Methods: Five poor metabolizers and 8 extensive metabolizers of debrisoquin (INN, debrisoquine) were given 10 mg selegiline hydrochloride. The concentrations of selegiline and its main metabolites in serum were determined for 4 days. The pharmacodynamics were quantitated by measuring platelet monoamine oxidase type B activity for 3 weeks. In addition, the effect of selegiline and its main metabolites on the CYP2D6-catalyzed dextromethorphan O-demethylase activity and the effect of quinidine on the metabolism of selegiline were studied in human liver microsomes.

Results: Peak serum concentrations of selegiline were reached rapidly and ranged from 1 to 32 nmol/L. The metabolite concentrations were considerably higher and remained so for a longer period. There were no significant differences in the pharmacokinetic parameters of selegiline, desmethylselegiline, and l-amphetamine between poor metabolizers and extensive metabolizers. However, the area under the serum concentration-time curve (AUC) values of l-methamphetamine were, on average, 46% higher (P < .01) in poor metabolizers than in extensive metabolizers. No significant correlations were found between debrisoquin metabolic ratio and AUC values of selegiline or its metabolites, except for l-methamphetamine (rs = 0.90; P l-methamphetamine toward dextromethorphan O-demethylase was very low (50% inhibitory concentration values from 160 to 580 [mu]mol/L). Quinidine (l-methamphetamine from selegiline.

Conclusions: CYP2D6 is not important in the primary elimination of selegiline, and the biological effect of selegiline seems to be similar in poor metabolizers and extensive metabolizers of debrisoquin. The inhibitory effect of selegiline and its main metabolites on CYP2D6 activity seems to be negligible.
mutations in the coding region (CYP2C9*2 and CYP2C9*3), mutations in the 5'-flanking region of the human CYP2C9 gene appear to contribute to the large interindividual variability in drug metabolism activity. (Clin Pharmacol Ther 2001;70:175-82.)


http://www.sciencedirect.com/science/article/B6WCN-4CRXGHG-8/2/af1acce29116a250b18ee88bdc9e6631

ObjectiveThe adenosine triphosphate-binding cassette transporter ABCG2 (breast cancer resistance protein [BCRP]) functions as an efflux transporter for many drugs, including the topoisomerase I inhibitor diflomotecan, and is expressed at high levels in the intestine and liver. We performed an exploratory analysis to evaluate the effects of the natural allelic variant ABCG2 421C>A on the pharmacokinetics of diflomotecan.

MethodsThe drug was administered to 22 adult white patients with cancer as a 20-minute infusion (dose, 0.10-0.27 mg), followed 2 weeks later by an oral solution (dose, 0.10-0.35 mg).

ResultsThe ABCG2 421C>A genotype significantly affected the pharmacokinetics of diflomotecan; in 5 patients heterozygous for this allele, plasma levels after intravenous drug administration were 299% (P =.015) of those in 15 patients with wild-type alleles, at mean values of 138 ng. h/mL. mg-1 (95% confidence interval, 11.3-264 ng. h/mL. mg-1) versus 46.1 ng. h/mL. mg-1 (95% confidence interval, 25.6-66.7 ng. h/mL. mg-1), respectively. Diflomotecan levels were not significantly influenced by 11 known variants in the ABCB1, ABCC2, cytochrome P450 (CYP) 3A4, and CYP3A5 genes.

ConclusionThese findings provide the first evidence linking variant ABCG2 alleles to altered drug exposure and suggest that interindividual variability in substrate drug effects might be influenced, in part, by ABCG2 genotype.


http://www.sciencedirect.com/science/article/B6WCN-49YGK44-P/2/419431acd232077be688af53b6bcede42

Objective: To evaluate the relationship between the metabolic ratio (MR) of metoprolol, CYP2D6*10B genotype, and the disposition of paroxetine in Korean subjects. Methods: A single 40-mg dose of paroxetine was administered orally to one poor metabolizer and 15 healthy subjects recruited from 223 Korean extensive metabolizers whose phenotypes were predetermined by use of the metoprolol MR. Genotypes were determined by allele-specific polymerase chain reaction and the GeneChip microarray technique. Pharmacokinetic parameters were estimated from plasma concentrations of paroxetine for more than 240 hours after the oral dose. Results: The oral clearance and area under the plasma concentration versus time curve (AUC) of paroxetine were best described by a nonlinear relationship with metoprolol MR at correlation coefficients of 0.82 and 0.91, respectively (P <.05). Nine extensive metabolizer who were either homozygous or heterozygous for CYP2D6*10B had significantly lower oral clearance values of paroxetine than six extensive metabolizers with CYP2D6*1/*1. The AUC of paroxetine in subjects who were homozygous for CYP2D6*10B (666.4 +/- 169.4 ng/mL. h) was significantly greater than that of subjects who were homozygous for the wild type (194.5 +/- 55.9 ng/mL. h). Unexpectedly, the average AUC of subjects who were heterozygous for CYP2D6*10B was greater with wide variation (789.8 +/- 816.9 ng/mL. h) than that of subjects who were homozygous CYP2D6*10B/*10B mainly because of two atypical subjects whose metoprolol MR was not associated with the CYP2D6*10B genotype and who showed greater AUC and lower oral
clearance than subjects with homozygous CYP2D6*10B. Conclusions: The CYP2D6 activity measured by metoprolol MR was a strong predictor of paroxetine disposition in Korean extensive metabolizers. In general, the extensive metabolizers with the CYP2D6*10B allele seemed to have higher plasma concentrations of paroxetine than extensive metabolizers with the wild-type CYP2D6 genotype. However, quantitative prediction of paroxetine disposition from the CYP2D6*10B genotype alone was not perfect because several Korean extensive metabolizers had metoprolol MRs that were not associated with the genotype. (Clin Pharmacol Ther 2000;67:567-76.)

Clinics in Dermatology  (1)


http://www.sciencedirect.com/science/article/B6T5G-45M5VNN-9/2/34f55f7a91f398a47a7ee742b1aed095

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology  (1)


http://www.sciencedirect.com/science/article/B6VNH-42D2CR1-8/2/00ac8deda34cc6939ca02776d319241d

Natriuretic peptide receptors in the central vasculature of the toad, Bufo marinus, were characterized using autoradiographical, molecular, and physiological techniques. Specific 125I-rat ANP binding sites were present in the carotid and pulmonary arteries, the lateral aorta, the pre- and post-cava, and the jugular vein, and generally occurred in each layer of the blood vessel. The 125I-rat ANP binding was partially displaced by the specific natriuretic peptide receptor C ligand, C-ANF, which indicates the presence of two types of natriuretic peptide receptors in the blood vessels. This was confirmed by a RT-PCR study, which demonstrated that guanylyl cyclase receptor (NPR-GC) and NPR-C mRNAs are expressed in arteries and veins. An in vitro guanylyl cyclase assay showed that frog ANP stimulated the production of cGMP in arterial membrane fractions. Physiological recordings from isolated segments of the carotid and pulmonary arteries and the lateral aorta, which had been pre-constricted with arginine vasotocin, showed that rat ANP, frog ANP and porcine CNP relaxed the vascular smooth muscle with relatively similar potency. Together, the data show that the central vasculature contains two types of natriuretic peptide receptors (NPR-C and NPR-GC) and that the vasculature is a target for ANP and CNP.

http://www.sciencedirect.com/science/article/B6T2P-3TGJRCW-4T/2/c94c96bea426368d19260277719e355c

The monophyly of the antarctic fish suborder Notothenioidei and the monophyly of its earliest family the Bovichtidae have been investigated with 12S and 16S mitochondrial DNA sequences. New data from Cottoperca, Pseudaphritis, Harpagifer and several outgroups, in addition to available sequences, show that the bovichtids are paraphyletic. Pseudaphritis is the sister group of all the non-bovichtid notothenioids. The same results are found from two independent genetic markers, the nuclear 28S rDNA and the 12S and 16S mitochondrial rDNA. This reliably refutes a previous hypothesis that placed Pseudaphritis as the sister group of all the remaining notothenioids (including Cottoperca and Bovichtus). Bootstrap analyses show that the Notothenioidei are monophyletic (although members of the suborder Trachinoidei have not been surveyed). Subsequent data from hemoglobin composition confirm the present relationships. After discussions between members of the European Science Foundation (ESF) network during its last two meetings, we point out here some fundamental aspects of comparative biology to improve understanding between the physiologist community and phylogeneticists. The most important points are differences in how the concept of homology is used and differences in the consideration of adaptation. When adaptation is evoked or questioned, endless speculations and untestable scenarios are often developed. We strongly advocate the use of phylogenetic trees for testing hypotheses of adaptation (through multiple character mapping). Such a "research program" in comparative biology has the power to improve knowledge because it can potentially lead to new experiments for testing adaptive hypotheses.


http://www.sciencedirect.com/science/article/B6T2R-43CCB4Y-8/2/f4b467e5f59b93266223439c30d00580

Transferrin was isolated from plasma of the ascidian Halocynthia roretzi by ion-exchange chromatography. The molecular weight of the plasma transferrin was determined to be 52K by SDS-polyacrylamide gel electrophoresis and gel filtration. Ascidian plasma transferrin was found to bind one mole of iron ion per mole of protein. The reductive S-pyridylethylated transferrin was subjected to Edman degradation analysis for determination of the N-terminal amino acid sequence, and it was also subjected to proteolytic fragmentation to yield peptide fragments, whose amino acid sequences were determined by Edman degradation analysis. Using the above amino acid sequences, a cDNA clone (1880 base pairs) encoding a protein of 372 amino acids
containing a signal peptide of 21 amino acids was isolated from an H. roretzi hepatopancreas cDNA library. The reduced amino acid sequence contains the same sequences of the peptide fragments. A comparison of the amino acid sequence of ascidian transferrin with those of other members of the transferrin family revealed that the ascidian transferrin is composed of only the N-terminal lobe of two-lobed vertebrate transferrins. Thus, a one-lobed transferrin is present in the ascidian H. roretzi.


http://www.sciencedirect.com/science/article/B6T2R-3WK3DWX-V/2/e9f40deab41fa0cf1d32538907f4dd39

A cDNA encoding a rainbow trout homologue of mammalian heart fatty acid binding protein (H-FABP) was isolated. The deduced protein sequence is 75% identical to that of rat H-FABP. The structural conservation of H-FABPs and their evolutionary relationship are discussed.


http://www.sciencedirect.com/science/article/B6T2R-43CCCV5-14/2/be8e449516bac67031dc4b60d0732a95

Using degenerative primers, partial cDNAs of a TNF (tumor necrosis factor) receptor and two TNF ligands were obtained by PCR of zebrafish and trout cDNAs, or cDNA libraries. These fragments were then used to screen cDNA libraries of appropriate tissues to obtain clones containing full coding sequences. A zebrafish cDNA was obtained that presumably codes for a 436 amino acid ovarian TNF receptor (OTR) that was identified as a death-domain-containing member of the TNF receptor family. On Northern blots, the OTR cDNA hybridized with a 3.4-kb transcript that is abundant in the zebrafish ovary but lightly detected in all other tissues tested. A zebrafish cDNA presumably coding for a 214 amino acid protein with sequence similarity to mammalian TRAIL (TNF-related apoptosis inducing ligand), was also isolated. In addition, a fragment of the brook trout TRAIL homologue was obtained. Finally, a full-length brook trout cDNA, that presumably codes for a 255 amino acid protein with sequence similarity to mammalian TNF-alpha and lymphotoxin-alpha, was isolated. This study is the first report of a death-domain-containing TNF receptor and the first published report of a TNF ligand in fish.


http://www.sciencedirect.com/science/article/B6T2R-492702J-1/2/f1239be88db22df50e6a8b3da3305516

The full-length growth hormone receptor (GHR) of gilthead sea bream (Sparus aurata) was cloned and sequenced by RT-PCR and rapid amplification of 5' and 3' ends. The open reading frame codes for a mature 609 amino acid protein with a hydrophobic transmembrane region and
all the characteristic motifs of GHRs. Sequence analysis revealed a 96 and 76% of amino acid identity with black sea bream (Acanthopagrus schlegeli) and turbot (Scophthalmus maximus) GHRs, respectively, but this amino acid identity decreases up to 52% for goldfish (Carassius auratus) GHR. By means of real-time PCR assays, concurrent changes in the hepatic expression of GHRs and insulin-like growth factor-I (IGF-I) was evidenced. Moreover, their regulation occurred in conjunction with the summer spurt of growth rates and circulating levels of GH and IGF-I. Search of alternative splicing was carried out exhaustively for gilthead sea bream GHR, but Northern blot and 3’ RACE failed to demonstrate the occurrence of short alternative messengers. Besides, RT-PCR screening did not reveal deletions or insertions that could lead to alternative reading frames. In agreement with this, cross-linking assays only evidenced two protein bands that match well with the size of glycosylated and non-glycosylated forms of the full-length GHR. If so, it appears that alternative splicing at the 3’end does not occur in gilthead sea bream, although different messengers for truncated or longer GHR variants already exist in turbot and black sea bream, respectively. The physiological relevance of this finding remains unclear, but perhaps it points out large inter-species differences in the heterogeneity of the GHR population.


http://www.sciencedirect.com/science/article/B6T2R-49F9WVK-2/2/69a2bd20bd9edf34e1ebbe08166d01a

Gene transcripts and enzyme activities were quantified for a selection of functionally important aminopeptidases at 2-day intervals throughout the first 72 days of development in the Pacific oyster Crassostrea gigas. Leucine aminopeptidase (LAP) and cathepsin B (CathB) gene transcripts were quantified using fluorogenic ('real time') PCR. LAP and CathB gene transcripts were detected at all time points. The proportion of CathB transcripts remained essentially constant and low throughout development (CtCtCt~23). CathB and cathepsin D (CathD) enzyme activities were measured biochemically. Whilst CathD activity peaked at day 19, LAP and CathB activities both peaked at day 24. The closely coupled increase in transcript and enzyme activity for LAP indicates regulation at the transcriptional level. Alternatively, the peak in enzyme activity for CathB without enhanced transcriptional activity suggests post-transcriptional regulation. Similar mechanisms of regulation for LAP and CathB have been observed in both plants and mammals, indicating widespread conservation.


http://www.sciencedirect.com/science/article/B6T2R-43CCCV5-1N/2/e54d30dade81f9a54ccf3c89f9fc94a1e1

Retinol-binding protein (RBP) is the specific carrier of retinol in vertebrates and forms a 1:1 complex with transthyretin (TTR). A cDNA encoding serum RBP was cloned from liver and 7-day larvae of the marine fish Sparus aurata. The mature protein is 176 amino acids long and shows sequence identity of 77-78%, 56%, 63% and 62% with rainbow trout, Xenopus, chicken and human RBP, respectively. Northern blot analysis of hepatic RBP revealed two transcripts: a major one of approximately 1.4-1.5 kb and a minor of approximately 0.7 kb. Distribution of RBP mRNA in various tissues was studied by RT-PCR and showed high expression in liver and skin, and low expression in brain, kidney and gill filament (20-35% of the level in liver). RBP expression in
intestine, pyloric caeca, muscle and pituitary was estimated to be approximately 7-14% of the level in liver. The ontogeny of RBP expression in S. aurata was examined in unfertilized eggs, embryos and larvae by using RT-PCR followed by hybridization with a specific probe. RBP transcript was found in all larval stages studied. Very low levels of RBP mRNA were detected in unfertilized eggs and in embryos 8 h after fertilization with a gradual increase at 12 h and 15-16 h post-fertilization. A single injection of estradiol-17[β] to S. aurata immature, bisexual fish or to adult males reduced steady-state levels of hepatic RBP by 37 and 25%, respectively. The same treatment induced vitellogenin expression. The present data suggest that in fish, liver is the main site of RBP synthesis, but that RBP may have an important function in fish skin. RBP is expressed early in embryonic development and in fish its expression can be down regulated by estrogen.


http://www.sciencedirect.com/science/article/B6T2R-4C1C7TW-5/2/748ed742a95c13624b924707b738014

The full-length cDNA for the cod (Gadus morhua) STAR was cloned by RT-PCR and library screening using ovarian RNA. From the library screening, 2 size classes of cDNA were obtained; a 1577 bp cDNA (cSTAR1) and a 2851 bp cDNA (cSTAR2). The cSTAR1 cDNA presumably encodes a protein of 286 amino acids. The cSTAR2 cDNA was composed of 6 separated sequences that contained all of the coding regions of cSTAR1 when added together, but also contained 5 noncoding regions not observed in cSTAR1. Polymerase chain reactions of cod genomic DNA produced products slightly larger than cSTAR2. The sequence of these products were the same as cSTAR2 but revealed one additional noncoding region (intron). Thus, the fish STAR gene contains the same number of exons (7) and introns (6) as observed in mammals, but is approximately half the size of the mammalian gene. Using Northern analysis and RT-PCR, cSTAR1 expression was observed only in testes, ovaries and head kidneys. Polymerase chain reaction products were also observed using cDNA from steroidogenic tissues and primers designed to regions specific for cSTAR2, indicating that cSTAR2 is expressed in tissues and may account for the presence of larger transcripts observed on Northern blots.


http://www.sciencedirect.com/science/article/B6T2R-3W3NDKC-W/2/99a55ea93e847ee6dacf2dd6044886b9

Recombinant mouse (Mus musculus) and rat (Rattus norvegicus) cystatin C were produced by expression in Escherichia coli, isolated and functionally characterized. The mouse and rat inhibitors were both fully active in titrations of papain. Determination of equilibrium constants for dissociation (Kᵢ) for their complexes with the target proteinase, cathepsin B, produced values not largely different from that for human cystatin C (Kᵢ 0.07-0.13 nM). Rabbit antisera against mouse and rat cystatin C were produced and used for improved affinity purification of the recombinant inhibitors. Affinity purified immunoglobulins isolated from the antiserum against mouse cystatin C were used for construction of a sensitive enzyme-linked immunosorbent assay. The assay was used to demonstrate a high degree of immunological cross-reactivity between mouse and rat cystatin C and could be used for cystatin C quantification in mouse and rat tissue homogenates. All tissues analyzed contained cystatin C, with a relative content very similar to that of human
tissues. For all species, brain tissue contained the highest cystatin C amounts and liver the lowest, whereas kidney, spleen and muscle tissues were intermediate in content. In the mouse, a notable high cystatin C content in parotid gland tissue was observed. The high degree of similarity in distribution pattern and functional properties for mouse, rat and human cystatin C indicates that: a murine model should be relevant for studies of the human disease, hereditary cystatin C amyloid angiopathy.


http://www.sciencedirect.com/science/article/B6T2R-3VB3FC1-P/2/56a977ba39055dc2a05bd8fe885e8472

The apparent high degree of homology of a blood protein with a unique dual binding affinity for two distinct hormones, thyroxin (T4) and vitamin D, isolated from a turtle, Trachemys scripta (Family Emydidae) and mammalian vitamin D binding protein (DBP) prompted further interspecific comparison to better understand the structure of functional binding sites. Using polymerase-chain reaction (PCR) with primers derived from the putative nucleotide sequences encoding peptides from the degradation of the T. scripta protein, we cloned the cDNA. The mature turtle protein contains 466 amino acids, about eight residues more than in mammalian DBP. The nucleotide sequence of the coding region showed 63% nucleotide and 73% amino acid homology ([ap]53% identity) to mammalian DBP (human, rat, mouse, and rabbit). However, there was no significant homology to mammalian T4-binding globulin (TBG) or transthyretin (TTR). Comparisons with mammals help define further the requirements for the vitamin D and actin binding sites. Northern blots of RNA isolated from turtle tissue probed with the 5' portion of cDNA established expression of the transcript in liver, kidney, and brain (in order of abundance), in contrast to mammal sequences in which expression of DBP is largely confined to the liver.


http://www.sciencedirect.com/science/article/B6T2R-408C90MB/2/0fb8fdda4a4edc36f0590808aec1644

A -galactose binding lectin (SLL-2) was isolated from Sinularia lochmodes, an octocoral, by a combination of affinity chromatography on acid-treated agarose and FPLC on Superdex 200. SLL-2 agglutinated rabbit and horse erythrocytes while SLL-1, a minor component, reacted only with rabbit erythrocytes. SLL-2 is a glycoprotein with a molecular mass of 122 kDa and is composed of eight identical subunits (15 kDa). The sequence of the amino terminal region of SLL-2 did not show any apparent homology to the sequences of other animal and plant lectins. -Galactose, N-acetyl-galactosamine, lactose, and melibiose were moderate inhibitors to the agglutination of rabbit erythrocytes. In contrast, horse erythrocytes were much more susceptible to agglutination by SLL-2, which was inhibited by sugars and glycoproteins such as -galactose, N-acetyl-galactosamine, lactose, melibiose, and porcine stomach mucin. SLL-2 showed considerable tolerance to heating and kept its activity after heating at 80[deg]C for 60 min. In immuno-histochemical studies using an anti-SLL-2 antiserum and protein A gold conjugate, SLL-2 was found to be present in high amounts in the nematocysts. SLL-2 was also detected on the surface of symbiotic dinoflagellate, Symbiodinium sp. cells irrespective whether they were surrounded with or without host cells. These observations suggest the presence of lectin-mediated interaction between symbiotic dinoflagellates and S. lochmodes.
The success of rainbow trout as an aquaculture species is dependent on the ability to produce fish with large amounts of high-quality lean muscle. It is therefore important to understand not only the best conditions under which to raise the fish but also the molecular control of muscle growth. Vertebrate muscle growth is initiated by the specification of myogenic precursor cells into myoblasts. The myoblasts proliferate and fuse to form multinucleated myotubes, which mature into myofibers. A family of basic helix-loop-helix (bHLH) transcription factors, the Myogenic Regulatory Factors (MRFs), controls these events. In trout, two MRF-encoding genes, TMyoD (of which there are two) and Tmyogenin, have been identified. However, the primary MRF-encoding Myf5 is not yet sequenced. Here, using degenerate PCR and 5' and 3' RACE, the cDNA sequence of trout Myf5 (TMyf5) is identified. Translation of the cDNA reveals that TMyf5 is a bHLH protein with homology to Myf5 and MRFs in other organisms. It is expressed mainly in red and white muscle, suggesting that it shares functional homology to Myf5 in other species. The molecular control of muscle growth has been well-characterized in mammals, but there are differences in the growth of fish muscle, highlighting the need for characterization of MRFs in fish species, particularly those in which understanding muscle growth will have a positive impact on the economic potential of the species.

Alternatively spliced isoforms of Na+-Ca2+ exchanger were found from various tissues and species. RT-PCR amplification was performed on the basis of our cloned mouse cardiac Na+-Ca2+ exchanger and four alternatively spliced isoforms of Na+-Ca2+ exchanger were identified. Three (NCX1.3, NCX1.4, and NCX1.12) of them were first identified in the heart, and one isoform (NCX1.12) was a novel spliced variant. These four spliced variants were present in the embryonic and adult atria and ventricles. Different cell types of the heart expressed different spliced isoforms of Na+-Ca2+ exchanger. Southern blot analysis indicated that the Na+-Ca2+ exchanger gene existed as a single copy in the mouse genome. Thus, the Na+-Ca2+ exchanger isoforms expressed in mouse heart are consistent with being produced by alternative splicing and they may have different functions in various cell types in the mouse heart.
cDNA fragments of both the [alpha]- and [beta]-subunits of the Na, K-ATPase and a cDNA fragment of the secretory form of Na-K-Cl cotransporter from the European dogfish (Scyliorhinus canicula) were amplified and cloned using degenerate primers in RT-PCR. These clones were used along with a sCFTR cDNA from the related dogfish shark, Squalus acanthias to characterise the expression of mRNAs for these ion transporters in the dogfish rectal gland subsequent to an acute feeding episode. Following a single feeding event where starved dogfish were fed squid portions (20 g squid/kg fish), there was a delayed and transient 40-fold increase in the activity of Na, K-ATPase in crude rectal gland homogenates. Increases in enzyme activity were apparent 3 h after the feeding event and peaked at 9 h before returning to control values within 24 h. These increases in activity were accompanied by small and transient decreases in plasma sodium and chloride concentrations lasting up to 3 days. Significant increases in the expression of mRNAs for [alpha]- and [beta]-subunits of the Na, K-ATPase, the Na-K-Cl cotransporter and CFTR chloride channel were detected but not until 1-2 days after the feeding event. It is concluded that the transient increase in Na, K-ATPase activity is not attributable to increases in the abundance of [alpha]- and [beta]-subunit mRNAs but must be associated with some, as yet unknown, post-transcriptional activation mechanism.


http://www.sciencedirect.com/science/article/B6T2R-40X8D55-W/2/e48e74efee413fde726ed820a895a84b

The fish otolith is a hard tissue consisting of calcium carbonate and organic matrices. The matrix proteins play important roles in otolith formation, but little is known about the nature of these proteins. In this study, matrix proteins were extracted from the otoliths of rainbow trout, Oncorhynchus mykiss, and chum salmon, Oncorhynchus keta. EDTA-soluble matrix proteins were separated by SDS-PAGE, revealing two major components in the otoliths of both species with apparent molecular masses of 55 and 43 kDa. N-terminal and some internal amino acid sequences of the 55-kDa otolith matrix protein were determined. A cDNA fragment encoding this protein of O. mykiss was amplified by reverse transcription PCR using two degenerate primers designed from the amino acid sequences. A cDNA encoding this protein was obtained by screening a saccular cDNA library using the amplified cDNA fragment as a probe. Nucleotide sequence analysis revealed that the cDNA clone has a sequence of 2.5 kb and the open reading frame encoding 344 amino acid residues. Northern blot analysis showed that mRNA of this protein is expressed specifically in the sacculus, and consistently during the day.


http://www.sciencedirect.com/science/article/B6T2R-3VB5X0-W/2/80f949892e87f778956e1831490183

The molecular weight of the liver-type subunit (L) of bovine ferritin is much larger than that of the heart-type subunit (H) as determined by SDS-PAGE (L, 20.5 kDa; H, 18.4 kDa). The migration of these two subunits on SDS-PAGE gels, relative to each other, is opposite to that reported for ferritin L and H subunits in other mammalian species (L, 19 kDa; H, 21 kDa). To determine the cause of this anomaly, full-length cDNA clones of the bovine L and H chains were isolated from a bovine spleen [lambda]gt11 cDNA library and sequenced. The amino acid sequences of the L and H chains of bovine ferritin, deduced from their cDNA sequences, contained open reading
frames coding for 174 and 180 amino acid residues with calculated molecular weights of 19,856 and 20,920 Da, respectively. The deduced amino acid sequence of the L chain shows 86%, 84%, 87%, 83% and 83% homology with the amino acid sequences of horse, human, rabbit, rat and mouse L chains, respectively. The H chain displays a higher homology with the human, rat and mouse H chains (91%, 92% and 93%, respectively). In addition, the bovine L chain did not contain the extra octapeptide present in rodent L chains, and bovine L and H chains did not react with concanavalin A. The bovine L and H chains expressed using a baculovirus expression system showed almost the same mobilities as those of bovine spleen ferritin, respectively, by SDS-PAGE. These results suggest that the much slower mobility of the bovine L chain compared with other mammalian L chains on SDS-PAGE cannot be attributed to insertion(s) of amino acid(s) or peptide(s) into the L chain, to the deletion(s) of them of it or to the addition of carbohydrate chain(s) but may result from significant differences in the binding affinity of SDS for bovine ferritin L chains.


http://www.sciencedirect.com/science/article/B6T2R-4B945XP-5/2/4f62be2bb1b204ec6710890f3553012c

Purified cathepsin L from carp, Cyprinus carpio, consists of a 28 kDa single-chain form that is different from the 24 and 5 kDa mammalian two-chain form. We cloned cathepsin L from carp hepatopancreas. The sequence consisted of a 1490 bp cDNA and a 1014 bp open reading frame, encoding a deduced protein of 337 amino acids that is likely processed to an active enzyme (single-chain form) with 222 amino acids. Its similarity to other types of vertebrate cathepsin L is less than 69%. Mammalian cathepsin L is further processed to a two-chain form, but possibly this is not the case with carp cathepsin L: the P1 site where cleavage occurred in the two-chain form of mammalian cathepsin L is a serine, while carp cathepsin L processes a valine. Therefore, carp cathepsin L may have a different mechanism of action from mammalian cathepsin L.


http://www.sciencedirect.com/science/article/B6T2R-3SG3M4Y-C/2/b8887aac95a7610c5421163d6d81d21

A bombyxin gene encoding precursor molecule for bombyxin-IV, one of the insulin-related neurosecretory peptide of the silkworm Bombyx mori, has been cloned and characterized. The nucleotide sequence of this gene and its deduced amino acid sequence deviate moderately from those characterized previously for the family A, B, C and D bombyxin genes. The gene encoding the bombyxin-IV precursor was therefore defined into a novel family E and designated as gene E1. The bombyxin E1 transcript in Bombyx brain was shown to locate in four pairs of medial neurosecretory cells, which also produce other bombyxin family mRNAs, and the amount of the E1 transcript did not change markedly during the fifth larval instar. Genomic Southern hybridization indicated that the Bombyx haploid genome contained a single copy of the bombyxin family E gene.

http://www.sciencedirect.com/science/article/B6T2R-43C5N6W-7/2/119b6f4d4206747868e776d22def314a

This study examined connexin (Cx) gene activity in relation to oocyte maturation in the red seabream (Pagrus major) ovary. Mixed primers for the polymerase chain reaction (PCR) were designed based on the high sequence homology of selected regions of known Cx genes. PCR-amplified cDNA fragments generated by 3’ and 5’ rapid amplification of cDNA ends (RACE) were combined to generate full-length cDNA sequences. The 1212-bp cDNA has an open reading frame encoding 282 amino acids, with a molecular mass of 32.3 kDa (red seabream Cx32.3). Hydropathy plots of red seabream Cx32.3 show the four typical major hydrophobic and four major hydrophilic regions of Cx proteins. Typical Cx consensus sequences are observed in the first and second extracellular loops. The ovarian follicles of matured female seabream were incubated in the presence of 17[alpha],20[beta]-dihydroxy-4-pregnen-3-one (DHP, 10 ng/ml), gonadotropin (GtH)-I (300 ng/ml) and GtH-II (300 ng/ml). Northern blot analysis of poly(A)+ RNA extracted from the ovarian follicles were hybridized with red seabream Cx32.3 and [beta]-actin probes. The transcription level of PmCx32.3 in the presence of DHP, PmGtH-I and PmGtH-II was significantly higher than in the control.

Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology (1)


http://www.sciencedirect.com/science/article/B6T2S-41TNDY4-M/2/de2b1b2c98a1101b2ea4d72f106948ae

Several studies have shown that the heart of species from each vertebrate class contains natriuretic peptide binding sites which suggests that ANP released from the heart may act in a paracrine/autocrine fashion. The present study used a set of techniques to study cell surface receptors in order to investigate the presence and nature of NPRs in the heart of the cane toad, Bufo marinus. Autoradiographical studies of both atria and ventricle showed no variation between total and non-specific binding, indicating a lack of NP binding sites in these tissues. This was confirmed with in vitro binding studies in which increasing concentrations of ANP did not compete for any specific binding. Increasing concentrations of ANP did not increase cGMP generation and physiological experiments showed that both ANP and CNP had no effect on the force or rate of contraction of a sino-atrial preparation. Molecular expression studies, however, showed that mRNA for NPRs was expressed in the heart, in spite of the lack of evidence for NPR on the cell surface. Overall, this study showed that no functional NPRs are present in the heart, and provided evidence that the heart is not a target organ for NP action in B. marinus.

http://www.sciencedirect.com/science/article/B6W89-4DF44HD-2/2/6f135f2e4df4ae8fd87d138a0e0ef67

Herein we report Xiphophorus DNA polymerase [beta] (XiphPol[beta]) mRNA and protein expression levels in brain, liver, gill, and testes tissues from Xiphophorus maculatus, Xiphophorus helleri, and Xiphophorus couchianus parental line fish and two different tumor-bearing Xiphophorus interspecies hybrids. Polymerase [beta] protein levels in the Xiphophorus tissues were measured by Western blot, and mRNA was measured with a quantitative real time RT-PCR method which employed cRNA construction to produce accurate calibration curves. We found significant differences in both mRNA and protein levels between the tumor-bearing hybrid animals and the three parental species. However, there were no significant differences in either mRNA levels or protein expression observed between the parental species. Thus, interspecies hybridization results in dysregulation of Pol[beta] expression and this may manifest a modulation in DNA repair capability and susceptibility to latent tumorigenesis.


http://www.sciencedirect.com/science/article/B6W89-4FHJYDV-3/2/81949daa546c8b1c2f675d423f072e88

Xenoestrogens such as 4-nonylphenol (4-NP) have been shown to affect the parr-smolt transformation, but their mechanisms of action are not known. We therefore examined effects of 4-NP and estradiol-17[beta] (E2) on expression of estrogen receptor (ER) [alpha] gene in the liver, gill, pituitary and brain of sockeye salmon to elucidate molecular mechanisms of 4-NP and E2 and developmental differences in response during smolting. Fish were treated twice within a week with 4-NP (15 and 150 mg/kg BW), E2 (2 mg/kg BW) or only vehicle at three stages of smolting, pre-smolting in March, early smolting in April and late smolting in May. The absolute amounts of ER[alpha] mRNA were determined by real-time PCR. The basal amounts of ER[alpha] mRNA peaked in April in the liver, gill and pituitary. In March, E2 extensively increased the amounts in the liver, while 4-NP had no effects at this stage. In contrast, 4-NP (but not E2) decreased liver ER[alpha] mRNA in April. 4-NP also decreased the amount of ER[alpha] mRNA in the gill in April. In the pituitary, 4-NP increased ER[alpha] mRNA in March but decreased it in May. There were no significant effects in the brain. Changes in basal ER[alpha] mRNA observed in this study indicate that estrogen responsiveness of tissues may change during salmon smolting. Furthermore, 4-NP and E2 have different effects on expression of ER[alpha] gene in the liver and gill during smolting, and the response is dependent on smolt stage.

Pituitary adenylate cyclase-activating peptide (PACAP), a novel compound with vasoactive intestinal polypeptide-like activity, was recently shown to be localized in the neuronal endings of the human uterus. The purpose of the present study was to assess the functional presence of PACAP mRNA in the decidual endometrium and its relationship to the expression levels of decidual prolactin-related protein (dPRP) and the progesterone receptor mRNAs during decidualization and pregnancy in Sprague-Dawley rats. PACAP was constitutively and temporally expressed in the decidual endometrium and gravid uterus. The time-dependent correlated expression levels of PACAP, dPRP and the progesterone receptor were induced by the neurogenic reproductive signals, i.e. the vagino-cervical/deciduogenic stimuli of decidualization and by the normal equivalent stimuli of mating/blastocyst implantation of gestation. Correlation among the mRNA expression levels of PACAP, dPRP and the progesterone receptor and the coordinated inhibitory actions of the anti-progesterone (RU-486) suggest that there is also correlated time-dependent steroid regulation of the mRNA levels of PACAP, dPRP and the progesterone receptor in the decidual and pregnant uteri. One possible functional meaning for the time-related localization of endometrial/uterine PACAP could be to facilitate endometrial blood flow and increase the availability of metabolic substrates to the developing deciduoma or embryo. The study demonstrates the potential importance of PACAP expression in the regulation of the maternal feto-placental component and suggests a prominent reproductive role for the neuropeptide in mammalian pregnancy.


As the immune system is known to be influenced by the endocrine system, the effects of hypophysectomy on immune functions were examined in the rainbow trout (Oncorhynchus mykiss). Superoxide anion (O2-) production, accompanied by phagocytosis, was significantly decreased in leucocytes isolated from the head kidney 7 days after hypophysectomy. Significant reduction was also observed in plasma immunoglobulin (Ig) M levels, whereas no change was observed in plasma lysozyme activity. The number of Ig-secreting leucocytes in peripheral blood had decreased after hypophysectomy, although total leucocyte number was not affected. The percentage of Ig-producing leucocytes as assessed by flow cytometry using a monoclonal antibody to trout IgM showed significant reduction in the head kidney. However, hypophysectomy did not affect the number of Ig-producing leucocytes in spleen, thymus or peripheral blood. By RT-PCR, expression of two growth hormones (GH I and II) and prolactin (PRL) mRNA was detected in lymphoid tissues, such as head kidney, spleen, thymus and intestine, as well as in leucocytes from blood and head kidney, indicating the local production of these hormones. These results indicate important roles of hypophyseal hormones produced not only in the pituitary, but also in the lymphoid tissues, in the maintenance of the immune functions in trout.

In vivo and in vitro effects of prolactin (PRL) and growth hormone (GH) on plasma levels of lysozyme and ceruloplasmin were examined in the rainbow trout (Oncorhynchus mykiss). Hypophysectomy had no effect on the plasma lysozyme level. Implantation of PRL- or GH-containing cholesterol pellets increased the lysozyme level in a dose-related manner. After hypophysectomy and sham operation, plasma ceruloplasmin was elevated above the level in intact fish, suggesting inflammation caused by the surgery. PRL or GH treatment significantly attenuated the increased level of ceruloplasmin in the operated fish. Expression of lysozyme mRNA was detected in the leucocytes isolated from the peripheral blood by RT-PCR. In vitro administration of PRL or GH showed no effect on the proliferation of isolated leucocytes or on the total protein content; however, lysozyme activity in the medium increased in a dose-related manner. These results suggest that PRL and GH directly stimulate lysozyme production without affecting the proliferation of leucocytes, and the attenuated ceruloplasmin level increased in response to inflammation.

Comparative Immunology, Microbiology and Infectious Diseases (7)


A polymerase chain reaction (PCR) technique was used to assay the presence of the aerolysin gene in a total of 89 Aeromonas hydrophila and A. sobria strains isolated from drinking water, fish and foods. These strains were also characterized for the production of virulence factors such as haemolysin, protease and cytotoxin. The primers used in the PCR targeted a 209-bp fragment of the aer gene coding for the [beta]-haemolysin and detected template DNA only in haemolytic A. hydrophila strains. The cell-free culture supernatants of these aerolysin-positive A. hydrophila strains were also cytotoxic to the HeLa and McCoy cells. The haemolytic A. sobria and non-haemolytic A. hydrophila were consistently negative in the PCR assay. Primer specificity was determined in the PCR by using a control haemolytic Escherichia coli, Streptococcus pyogenes and a restriction endonuclease assay. The PCR clearly identified the aerolysin-producing strains of A. hydrophila and may have application as a rapid species-specific virulence test.


http://www.sciencedirect.com/science/article/B6T5H-45XR89W-1/2/b836789fd99cbcd03f053aba3ffce5b8


http://www.sciencedirect.com/science/article/B6T5H-3WK3RT6-1/2/086df03f6f7877d35002768d34d91081

E. coli strains isolated from pigs with postweaning diarrhea or edema disease were tested by phenotypic and genotypic methods for the presence of virulence antigens and genes, respectively. The slide agglutination and ELISA analyses were used for determination of F4, F5, F6, F17, and F41 fimbriae whereas the prevalence of fimbrial fedA and toxin eltI, estI, estII, stx1, stx2 and stx2e genes were recorded by the means of PCR. Only F4 antigen (ac variant) was found in strains of the serogroup O149:K91 isolated from pigs with diarrhea. PCR analyses showed that the fedA gene encoding F18 fimbriae was present in 61.9% of strains isolated from pigs with diarrhea and in 84.2% of strains isolated from pigs with edema disease. The eltI genes encoding heat-labile toxin I (LTI) were present only in 9 out of 21 strains recovered from pigs with diarrhea. Shiga toxin 2 variant (stx2e) genes were found in six isolates from edema disease and also in one strain from diarrhea. The PCR test used in the study was a sensitive and valuable method for determination of virulence factors of E. coli strains.


http://www.sciencedirect.com/science/article/B6T5H-487MTS9-2/2/dffdeccbb3524b2dd63e79c83ff5c24d27

Three goats from a group of five caprine herpesvirus 1 (CpHV.1) seronegative pregnant goats were inoculated intranasally with a virulent BA.1 strain of CpHV.1. Goat n.1 was infected on day 45 of pregnancy, goat n.2 on day 92 and goat n.3 on day 127. Each of the three goats produced a single foetus 10-60 days after infection. Foetus n.1 was never found and so it could not be examined for virological findings. Goat n.2 delivered at term of gestation and CpHV.1 was detected by PCR and isolated from most of the foetal organs. Foetus n.3 was partially autolysed and the virus was only detected by PCR but not isolated from foetal organs. The results confirm the damaging effect of CpHV.1 infection on pregnancy, the difficulty in diagnosing the CpHV.1 induced abortion, and the importance developing appropriate prophylactic programmes.


http://www.sciencedirect.com/science/article/B6T5H-3VM10K4-5/2/80a63d5b374e1b8020fd7df3bbc242de
Inhibition of isolation of Listeria monocytogenes by bacteriocin-like substance (BLS)-producing Listeria innocua after enrichment culture was investigated. When 26 L. monocytogenes strains were examined in combination with eight L. innocua strains using the spot on lawn method, 52/208 (25.0%) combinations showed the growth inhibition of L. monocytogenes. When two Listeria species were cultured simultaneously in selective enrichment broth, inhibition of isolation of L. monocytogenes was observed in 12/52 of the combinations at 24 h (23.1%), in 24/52 at 48 h (46.2%) and in 30/52 (57.7%) after 7 days of incubation. The randomly amplified polymorphic DNA profiles showed no interstrain similarities between either strains of the BLS-producing L. innocua or the BLS-sensitive L. monocytogenes strains. Therefore inhibition by BLS-producing L. innocua of isolation of L. monocytogenes after enrichment culture is unlikely to be dependent upon a particular genetic profile.

**Resume**
L'inhibition de l'isolement de Listeria monocytogenes par une substance telle que la bacteriocine (BLS) produisant de la Listeria innocua aprés une culture enrichie fut étudiée. Lorsque 26 souches de L. monocytogenes furent examinées en combinaison avec 8 souches de L. innocua en utilisant la plaque d'une méthode de tapis, 52 combinaisons sur 208 (25.0%) montrèrent une inhibition croissante de L. monocytogenes. Lorsque 2 variétés de Listeria furent cultivées simultanément dans un bouillon enrichi sélectif, une inhibition de l'isolement de L. monocytogenes fut observée dans 12 combinaisons sur 52 en 24 h (23.1%), 24 sur 52 en 48 h (46.2%) et 30 sur 52 (57.7%), aprés 7 jours d'incubation. Les profils d'une amplification aleatoire polymorphique de l'ADN ne montrèrent pas de similarités intersouches entre les souches de BLS produisant de la L. innocua ou les souches sensibles BLS de L. monocytogenes. Par conséquent, une inhibition par BLS produisant de la L. innocua provenant de l'isolement de L. monocytogenes aprés une culture enrichie est improbable, selon qu'il s'agit d'un profil génétique particulier.

**Comptes Rendus Biologies**


We describe a high-throughput cDNA sequencing pipeline (http://www.hgsc.bcm.tmc.edu/projects/cdna) built in response to the emerging need for rapid sequencing of large cDNA collections. Using this strategy cDNA inserts are purified and joined through concatenation into large molecules. These 'pseudo-BACs' are subjected to random shotgun sequencing whereby the majority of cDNA inserts in the pool are sequenced. Using this concatenation cDNA sequencing platform, we have contributed more than 13 000 full-length cDNA sequences from human and mouse to the Mammalian Gene Collection (MGC). To cite this article: P.H. Gunaratne et al., C. R. Biologies 326 (2003).

http://www.sciencedirect.com/science/article/B6X1F-49H69XT-5/2/9f49f52a1f3108106c8198e2a8ad3b86

In order to investigate human-louse phylogeny, we partially sequenced two nuclear (18S rRNA and EF-1[alpha]) and one mitochondrial (COI) genes from 155 Pediculus from different geographical origins. The phylogenetic analysis of 18S rRNA and EF-1[alpha] sequences showed that human lice were classified into lice from Sub-Saharan Africa and lice from other areas. In both clusters, head and body lice were clearly grouped into two separate clusters. Our results indicate that the earliest divergence within human pediculidae occurred between African lice and other lice, and the divergence between head and body lice was not the result from a single event. To cite this article: Z. Yong et al., C. R. Biologies 326 (2003).


http://www.sciencedirect.com/science/article/B6VJ5-402TP37-1/2/b13dc6d1eeec86d58d6e1af72b9b0a800

We describe here a new stop mutation at triosephosphate isomerase (TPI) position 145 in a Hungarian family for which the first mutation (240 Phe -> Leu) was published earlier. The entire genomic TPI locus (exons, introns and promoter) was sequenced and found to be identical in the two compound-heterozygote brothers. Both brothers have the same well-compensated level of non-spherocytic hemolytic anemia and very high levels of the TPI substrate dihydroxyacetonephosphate (DHAP), but only one brother manifests neurologic disorders. Differences in nonsense-mediated mRNA decay may be at the basis of the differences in phenotype expression although it cannot be excluded the interaction with a modifier gene. Based on our earlier results, the development of neurodegeneration may be decisively modulated by the cellular environment of the mutant proteins initiating the process of focal apoptosis of neurons in glycolytic, peroxisomal and prion-induced neurological diseases.

Empirical assessments of transgene inheritance and phenotypic expression will assist in the development of efficient breeding strategies for transgenic germplasm, and guide research into the improvement of transformation techniques. The inheritance of a barley yellow dwarf virus (BYDV) coat protein gene and bar, and the expression of bar as measured by resistance to glufosinate-ammonium damage, was studied in the T1 and T3 generations of barley (Hordeum vulgare L.) populations derived from seven independent transformation events. Most populations deviated from Mendelian inheritance patterns, and several showed evidence of transgene silencing. To further study transgene behavior, several transgenic lines were crossed to a diverse set of nontransgenic cultivars and breeding lines to produce single cross- and backcross-derived populations. In these populations, the inheritance of glufosinate-ammonium resistance generally fit Mendelian expectations for single, dominant loci. Quantitative measurements of glufosinate-ammonium resistance showed heritable variability for glufosinate-ammonium resistance both among and within individual transformation events, but no variability could be attributed to the different genetic backgrounds of the nontransgenic parents. It is concluded that, although transgenic parents such as these can be used in a breeding program, transformation systems that result in greater stability of transgene behavior are desirable.


Reciprocal recurrent selection (RRS) in maize (Zea mays L.) is used to develop populations with superior combining ability, and it is usually assumed that these populations must be genetically distinct for RRS to be effective. Starting from two randomly derived subpopulations, GG(A) and GG(B), of the open-pollinated maize population Golden Glow’ (GGC0), we conducted six cycles of full-sib RRS for grain yield and moisture. Our objectives were to (i) document selection response; (ii) evaluate inter and intrapopulation genetic diversity; and (iii) review selection response relative to gene action, heterosis, inbreeding, and genetic drift. We performed a generation means analysis (GMA) using GG(A), GG(B), and a third population of Golden Glow developed by 21 cycles of mass selection for prolificacy, GG(MP). Field performance was evaluated at four environments in Wisconsin. We analyzed allele frequency changes using simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs). Selection response for grain yield of GG(A) x GG(B) population crosses was 5.3 g plant-1 cycle-1. Grain moisture and root lodging decreased by -0.5% cycle-1 and -5.2% cycle-1, while prolificacy increased by 0.03 ears cycle-1. After six cycles of RRS, GG(A) and GG(B) diverged genetically from each other, as well as from GG(MP)C21 and Golden Glow. Gene diversity within GG(A) and GG(B) decreased. However, total gene diversity over both GG(A) and GG(B) did not change over cycles of RRS. Genetic drift did not appear to seriously impede selection response. Most of the selection response, regardless of trait, selection method, or subpopulation, was attributed to additive genetic effects.

this study was to evaluate the genetic diversity and yield of families derived from crosses between northern elite (NE) by southern elite (SE) parents. Lines were derived from 10 crosses of NE x SE parents. Molecular markers were used to estimate genetic distance between each line and its SE parent. Yield and agronomic traits were measured in field trials from 1997 to 1999 in six of the crosses. The association of diversity with line yield, expressed relative to yield of the SE parent was determined with regression. On average, the use of NE parents reduced yield, relative to using other SE parents. Some crosses and NE parents were better than others and produced families with yield that exceeded that of their SE parent, indicating that some genes from the NE parents were superior to the genes in the SE parent. At least one line with yield either superior or similar to their SE parent was found in each cross. The finding of positive transgressive segregants in some crosses and the results of the regression analyses indicate that most of the NE parents possess some yield genes that are likely to be superior to those of the SE parents. Our approach to selecting for diversity and yield may be applicable to large introgression programs where diversity from many sources is desired.


Rust resistance genes Lr37, Sr38, and Yr17 are located within a segment of Triticum ventricosum (Tausch) Cess. chromosome 2NS translocated to the short arm of bread wheat chromosome 2AS. Characterization of this chromosome segment by 13 restriction fragment length polymorphism (RFLP) markers indicated that the 2NS translocation replaced approximately half of the short arm of chromosome 2A (distal 25-38 centimorgans, cM). The objective of this study was to develop polymerase chain reaction (PCR) assays based on RFLP marker cMWG682 to facilitate the transfer of this cluster of rust resistance genes into commercial wheat (Triticum aestivum L.) cultivars. DNA sequence was obtained from the A-, B-, D-, and N-alleles of cMWG682 and was used to design N-allele specific primers. The 2NS fragment amplified by PCR primers cosegregated with the presence of the RFLP-2NS band in all backcross populations. A cleaved amplified polymorphic sequence (CAPS) was used to develop a marker for the 2A-allele. This marker can be used to differentiate homozygous and heterozygous plants carrying the 2NS translocation in the final cycle of backcross introgression or in screenings for homozygous plants in segregating populations. Finally, a third PCR assay was developed by means of TaqMan technology as a high-throughput alternative for selection of the 2NS/2AS translocation in large segregating populations in breeding programs that have access to real time PCR equipment. These molecular markers were used to develop four hard red spring isogenic lines homozygous for the 2NS chromosome segment. One of the isogenic lines, derived from Anza,’ did not show the expected resistance in spite of the presence of all the RFLP markers for the 2NS chromosome segment. Analysis of F1 hybrids suggested that a suppressor of the Lr37 gene is present in Anza. These isogenic lines will provide a valuable tool to test the effects of the large 2NS translocation on quality and agronomic performance.


Seed size is an important attribute of soybean [Glycine max (L.) Merr.] for some food uses. The objectives of this study were to identify simple-sequence-repeat (SSR) markers associated with quantitative trait loci for seed size (SSQTL) and to compare the effectiveness of phenotypic
Selection and marker-assisted selection for seed size among individual F2 plants. Three small-seeded lines were crossed to parents with normal seed size to form three two-parent populations. The parents of the populations were screened with 178 SSR markers to identify polymorphism. Population 1 (Pop 1) had 75 polymorphic SSR markers covering 1306 centimorgans (cM), Pop 2 had 70 covering 1143 cM, and Pop 3 had 82 covering 1237 cM. Seed size of each population was determined with 100 F2 plants grown at Isabela, Puerto Rico, and their F2-derived lines grown in two replications at three environments. Single-factor analysis of variance and multiple regression were used to determine significant marker-SSQTL associations. Population 1 had 12 markers that individually accounted for 8.1 to 14.9% of the variation for seed size combined across environments, Pop 2 had 16 markers that individually accounted for 7.8 to 36.5% of the variation, and Pop 3 had 22 markers that individually accounted for 8.6 to 28.8% of the variation. Three marker loci that had significant SSQTL associations in this study also were significant in previous research, and 13 markers had unique SSQTL associations. The relative effectiveness of phenotypic and marker-assisted selection among F2 plants varied for the three populations. Averaged across the three populations, phenotypic selection for seed size was as effective and less expensive than marker-assisted selection.


http://crop.scijournals.org/cgi/content/abstract/44/1/265

Soybean [Glycine max (L.) Merr.] germplasm PI 437654 exhibits broad resistance to soybean cyst nematode (Heterodera glycines Ichinohe, SCN). Probes derived from PI 437654 bacterial artificial chromosome (BAC) clone 15G19 at the Rhg4 locus detected a restriction fragment length polymorphism (RFLP) between resistant and susceptible germplasms. Detailed RFLP analysis using restriction fragments from BAC clone 15G19 associated the polymorphism with an 8-kb BamHI fragment containing the promoter region and partial coding sequence of a novel soybean subtilisin-like protease, GmSUB1. Complete sequence of GmSUB1 was determined (GenBank AY277949). Regulatory elements for root gene expression, pathogen response, coordinated multiple-gene expression, and a novel 90-bp direct repeat were identified. GmSUB1 shows 74% similarity to Arabidopsis thaliana AIR3. Hybridization analyses indicate that PI 437654 contains only full-length copies of GmSUB1, whereas susceptible germplasm Williams 82 contains both full-length and truncated copies of the gene. A 4-fold increase in GmSUB1 copy number, and a corresponding 2- to 3-fold increase in steady state GmSUB1 mRNA levels, was observed in PI 437654 compared with Williams 82. Localization and polymorphism of GmSUB1 within the Rhg4 resistance region, and increases in GmSUB1 gene copy number and expression in PI 437654 compared with Williams 82 infers a functional role in the pathogen response. GmSUB1 is believed to be secreted into the extracellular matrix, and may function in reorganization of cell wall components during plant development and in the defense response.


http://crop.scijournals.org/cgi/content/abstract/43/4/1506

A critical impediment to field testing and deployment of transgenic sorghum [Sorghum bicolor (L.) Moench] is the threat of gene flow to weedy relatives through pollen. A technique using sorghum with A3 cytoplasmic male sterility to control transgene flow through pollen while using nontransgenic pollinators is described and an experiment was designed to evaluate the risk of viable pollen flow using A3 hybrids under field conditions. Seed set under pollinating bags (an indicator of fertile pollen) was evaluated at the University of Nebraska Field Laboratory at Ithaca,
NE, in 2001 and 2002 on selfed F2 progeny grown from open pollinated seed of 12 F1 hybrids produced in A1 and A3 cytoplasm. The F2 seed was produced in hybrid yield trials in 1997 and 1998 at Ithaca, NE. In each evaluation year, the experimental design was a split-split plot with seed production year the main plot factor, hybrid as the subplot factor, and cytoplasm as the sub-subplot factor. Cytoplasm effects were highly significant, with percent seed set on A1 F2 individuals averaging 74%, and on A3 F2 individuals averaging 0.04%. Upper confidence limits (P = 0.05) for percent seed set were 1.32% or less for the progeny from all A3 hybrids. Polymerase chain reaction (PCR) analysis confirmed that four male fertile individuals (from a population of 1007) were detected with A3 cytoplasm. These results support the hypothesis that gene flow through pollen can be severely restricted but not eliminated in sorghum by the use of A3 cytoplasmic male sterility.


http://crop.scijournals.org/cgi/content/abstract/42/5/1451

This study tests the genetic difference between landrace populations of *Solanum tuberosum* L. subsp. *tuberosum* and subsp. *andigenum* (Juz. & Bukasov) Hawkes using nuclear DNA microsatellites. Microsatellite loci were amplified in subsp. *andigenum* (35 accessions), subsp. *tuberosum* (35 accessions), and other cultivated and wild species (22 accessions). A total of 208 alleles were scored from 18 microsatellite loci spread throughout all 12 chromosomes of potato. Using an infinite allele model and a least squares method of analysis, microsatellite loci separated subsp. *tuberosum* from subsp. *andigenum*, and cultivated and wild species. These results support the genetic difference of these two populations and their recognition at some classification level.


http://crop.scijournals.org/cgi/content/abstract/44/3/963

To determine if standard breeding methodology is applicable to transgenes, phenotypic recurrent selection was used to select for increased transgene expression in white clover, *Trifolium repens* L. Plants were transformed with nptII and gusA, and selected on 100 mg L-1 of kanamycin. Independently transformed plants were intercrossed, and the progeny was germinated on 200, 300, or 400 mg L-1 of kanamycin. Those seedlings surviving on 400 mg-1 were in turn intercrossed, and the progeny was selected on 300, 400, or 500 mg L-1 of kanamycin. NPTII levels were measured in each selected population, and Southern blots were made from individuals in each population. The highest-expressing individual in the T2 had levels of NPTII that were more than four times higher than those in the highest parent. With selection on increasing levels of kanamycin, average expression across each generation went from 0.033 ng {micro}g-1 NPTII in the parents to 0.095 ng {micro}g-1 in the selected T1 plants to 0.539 ng {micro}g-1 in the selected T2 plants. Southern hybridization suggested that plants displaying a heightened level of nptII expression in the T1 and T2 fell into two categories. The first contained one particular transgenic event, implicating the importance of other genomic factors in modulating gene expression. Alternatively, the plants had an accumulation of various nptII loci, suggesting an association between multiple transgene copies and high expression levels. On the basis of these results, selection for transgene expression appears to be a viable option for plant breeding programs.
Genetic improvement for yield in soybean [Glycine max (L.) Merr.] has been accomplished by breeding within a narrow elite gene pool. Plant introductions (PIs) may be useful for obtaining additional increases in yield if unique and desirable alleles at quantitative trait loci (QTL) can be identified. The objectives of the study were to identify QTL for yield in elite and PI germplasm and to determine if the PIs possessed favorable alleles for yield. Allele frequencies were measured with simple sequence repeat (SSR) markers in three populations, designated AP10, AP12, and AP14, that differed in their percentage of PI parentage. AP10 had 40 PI parents, AP12 had 40 PI and 40 elite parents, and AP14 had 40 elite parents. Four cycles of recurrent selection for yield had been conducted in the three populations. Allele frequencies of the highest-yielding C4 lines in the three populations were compared with the parents used to form the populations of the initial cycles. Allele flow was simulated to account for genetic drift. Fifty-four SSRs were associated with 43 yield QTL. Seven of the QTL had been identified in previous research. Sixteen favorable marker alleles were unique to the PI parents. The genes associated with the unique PI alleles merit further investigation for their potential to increase yield of soybean cultivars.

Current Biology (9)


Background: Heavy-chain diseases (HCDs) are human lymphoproliferative neoplasias that are characterized by the secretion of truncated immunoglobulin heavy chains devoid of light chains. We have previously proposed -- by analogy to the process by which mutated growth factor receptors can be oncogenic -- that because the genetic defects in HCDs result in the production of abnormal membrane-associated heavy chains lacking an antigen-binding domain, these abnormal B-cell antigen receptors might engage in ligand-independent signalling. Normal pre-B-cell development requires the presence of the pre-B-cell receptor, formed by the association of [mu] heavy chains with two polypeptides -- so-called surrogate light chains, Vpre-B and [lambda]5 -- that are homologous to the variable and constant portions of immunoglobulin light chains, respectively. To assess whether amino-terminal truncation of membrane-associated heavy chains results in their constitutive activation, we have examined the ability of a HCD-associated [mu] protein to promote pre-B-cell development in transgenic mice. Results When the [mu] HCD transgene is introduced into SCID mice, CD43- pre-B cells develop normally. To determine whether this pre-B-cell development requires surrogate light chains, we backcrossed mice expressing full-length or truncated [mu] transgenes with [lambda]5-deficient mice. Our results show that the truncated heavy chain, but not the normal chain, is able to promote pre-B-cell development in the absence of [lambda]5. We also show that truncated [mu] chains spontaneously aggregate at the surface of bone marrow cells. Conclusion Expression of the truncated [mu] heavy chain overrides a tightly controlled step of pre-B-cell development, which
strongly suggests that a constitutive signal is delivered by the truncated [mu] chain disease protein. The self-aggregation of [mu] chain disease proteins might account for this constitutive activation. We conclude that amino-terminal truncation of heavy chains could play a role in the genesis of HCD neoplasia if it occurs at an appropriate stage of B-cell differentiation, namely in a mature B cell.


http://www.sciencedirect.com/science/article/B6VRT-4CB6PVS-6/2/9aaceee2345f4b6d676e924c8a3d8878d

Background: Variants of the green fluorescent protein (GFP) with different colors would be very useful for simultaneous comparisons of multiple protein fates, developmental lineages and gene expression levels. The simplest way to shift the emission color of GFP is to substitute histidine or tryptophan for the tyrosine in the chromophore, but such blue-shifted point mutants are only dimly fluorescent. The longest wavelengths previously reported for the excitation and emission peaks of GFP mutants are 488 and 511 nm, respectively. Results: Additional substitutions, mainly in residues 145-163, have improved the brightness of the blue-shifted GFP mutants with histidine and tryptophan in place of tyrosine 66. Separate mutations have pushed the excitation and emission peaks of the most red-shifted mutant to 504 and 514 nm, respectively. At least three different colors of GFP mutants can now be cleanly distinguished from each other under the microscope, using appropriate filter sets. A fusion protein consisting of linked blue- and green-fluorescent proteins exhibits fluorescence resonance energy transfer, which is disrupted by proteolytic cleavage of the linker between the two domains. Conclusion: Our results demonstrate that the production of more and better GFP variants is possible and worthwhile. The production of such variants facilitates multicolor imaging of differential gene expression, protein localization or cell fate. Fusions between mutants of different colors may be useful substrates for the continuous in situ assay of proteases. Demonstration of energy transfer between GFP variants is an important step towards a general method for monitoring the mutual association of fusion proteins.


http://www.sciencedirect.com/science/article/B6VRT-428DKHP-1/2/763173edc9cc475e17a77f185bae97fa

Background: In the leech Helobdella robusta, an annelid worm, the early pattern of cell divisions is stereotyped. The unequal first cleavage yields cells AB and CD, which differ in size, cytoplasmic inheritance, normal fate, and developmental potential. Results: Here we report a dynamic and transcription-independent pattern of WNT signaling in the two-cell stage of H. robusta. Surprisingly, HRO-WNT-A is first expressed in a stochastic manner, such that either AB or CD secretes the protein in each embryo. This stochastic phase is followed by a deterministic phase during which first AB, then CD expresses HRO-WNT-A. When contact between the cells is reduced or eliminated, both AB and CD express HRO-WNT-A simultaneously. Finally, bathing embryos in anti-HRO-WNT-A antibody during first cleavage reduces the adhesion between cells AB and CD. Conclusions: Our findings show that the stochastic phase of HRO-WNT-A signaling in the two-cell stage of Helobdella is negatively regulated by cell-cell contact and that this early signaling affects cell adhesion without affecting cell fate. We speculate that the primordial function of wnt class genes may have been to regulate cell-cell adhesion and that the nuclear signaling components of the wnt pathway arose later in association with the evolution of diverse cell types.

http://www.sciencedirect.com/science/article/B6VRT-41N57JK-1D/2/6db4159551b6da3b025df3415c9f1920

Background: Two competing hypotheses for the origins of Polynesians are the 'express-train' model, which supposes a recent and rapid expansion of Polynesian ancestors from Asia/Taiwan via coastal and island Melanesia, and the 'entangled-bank' model, which supposes a long history of cultural and genetic interactions among Southeast Asians, Melanesians and Polynesians. Most genetic data, especially analyses of mitochondrial DNA (mtDNA) variation, support the express-train model, as does linguistic and archaeological evidence. Here, we used Y-chromosome polymorphisms to investigate the origins of Polynesians.

Results: We analysed eight single nucleotide polymorphisms (SNPs) and seven short tandem repeat (STR) loci on the Y chromosome in 28 Cook Islanders from Polynesia and 583 males from 17 Melanesian, Asian and Australian populations. We found that all Polynesians belong to just three Y-chromosome haplotypes, as defined by unique event polymorphisms. The major Y haplotype in Polynesians (82% frequency) was restricted to Melanesia and eastern Indonesia and most probably arose in Melanesia. Coalescence analysis of associated Y-STR haplotypes showed evidence of a population expansion in Polynesians, beginning about 2,200 years ago. The other two Polynesian Y haplotypes were widespread in Asia but were also found in Melanesia.

Conclusions: All Polynesian Y chromosomes can be traced back to Melanesia, although some of these Y-chromosome types originated in Asia. Together with other genetic and cultural evidence, we propose a new model of Polynesian origins that we call the 'slow-boat' model: Polynesian ancestors did originate from Asia/Taiwan but did not move rapidly through Melanesia; rather, they interacted with and mixed extensively with Melanesians, leaving behind their genes and incorporating many Melanesian genes before colonising the Pacific.


http://www.sciencedirect.com/science/article/B6VRT-49BYF2J-S/2/0c8e3bd4e7de24a2032696b240067a33

The human head louse (Pediculus humanus capitis) and body louse (P. humanus corporis or P. h. humanus) are strict, obligate human ectoparasites that differ mainly in their habitat on the host [1 and 2]: the head louse lives and feeds exclusively on the scalp, whereas the body louse feeds on the body but lives in clothing. This ecological differentiation probably arose when humans adopted frequent use of clothing, an important event in human evolution for which there is no direct archaeological evidence. We therefore used a molecular clock approach to date the origin of body lice, assuming that this should correspond with the frequent use of clothing. Sequences were obtained from two mtDNA and two nuclear DNA segments from a global sample of 40 head and body lice, and from a chimpanzee louse to use as an outgroup. The results indicate greater diversity in African than non-African lice, suggesting an African origin of human lice. A molecular clock analysis indicates that body lice originated not more than about 72,000 +/- 42,000 years ago; the mtDNA sequences also indicate a demographic expansion of body lice that correlates with the spread of modern humans out of Africa. These results suggest that clothing was a surprisingly recent innovation in human evolution.
Background: Human epidermis is renewed throughout life from stem cells in the basal layer of the epidermis. Signals from the surrounding keratinocytes influence the differentiation of the stem cells, but the nature of the signals is unknown. In many developing tissues, signalling mediated by the transmembrane protein Delta1 and its receptor Notch1 inhibits differentiation. Here, we investigated the role of Delta-Notch signalling in postnatal human epidermis.

Results: Notch1 expression was found in all living epidermal layers, but Delta1 expression was confined to the basal layer of the epidermis, with highest expression in those regions where stem cells reside. By overexpressing Delta1 or DeltaT, a truncated form of Delta1, in primary human keratinocytes and reconstituting epidermal sheets containing mixtures of Delta-overexpressing cells and wild-type cells, we found that cells expressing high levels of Delta1 or DeltaT failed to respond to Delta signals from their neighbours. In contrast, wild-type keratinocytes that were in contact with neighbouring cells expressing Delta1 were stimulated to leave the stem-cell compartment and initiate terminal differentiation after a few rounds of division. Delta1 promoted keratinocyte cohesiveness, whereas DeltaT did not.

Conclusions: We propose that high Delta1 expression by epidermal stem cells has three effects: a protective effect on stem cells by blocking Notch signalling; enhanced cohesiveness of stem-cell clusters, which may discourage intermingling with neighbouring cells; and signalling to cells at the edges of the clusters to differentiate. Notch signalling in epidermal stem cells thus differs from other progenitor cell populations in promoting, rather than suppressing, differentiation.

Background: Many site-specific recombinases act by forming and resolving branched Holliday junction intermediates. Previous findings have been consistent with models involving branch migration across the ‘overlap region’ of obligate homology, located between the staggered sites where the two single-strand exchanges occur. We have investigated the validity of such models in the case of bacteriophage [lambda] site-specific recombination.

Results By using synthetic [lambda] att-site Holliday junctions, incorporating sequence heterologies that impose constraints on branch migration, we have found that the optimal position of the junction for either top-strand or bottom-strand resolution by [lambda] integrase (Int) is not at the ends, but close to the middle of the seven base-pair overlap region. A minor shift of the branch point around the central base pair caused a remarkable switch in resolution bias. Our findings suggest that branch migration is limited to the central one to three base pairs of the overlap region. They lead to a new model for [lambda] site-specific recombination, in which there are two symmetrical swaps of two to three nucleotides each, linked by a central isomerization step that causes a change of the stacking interactions between the four junction arms. On the basis of isolated strand-joining reactions carried out by Int in the presence or absence of base complementarity, we propose that sequence homology is sensed during the annealing step prior to strand joining. The new model eliminates mechanistic complications associated with large helical rotations required by branch-migration models.

Conclusion The results reported here suggest that the recognition of sequence homology in Int-dependent site-specific recombination does not rely primarily on branch migration. The property of cleaving Holliday junctions a few base pairs away from the crossover puts [lambda] Int into the same category as endonucleases that cleave Holliday junctions in homologous recombination.
The determination of nuclear DNA sequences from ancient remains would open many novel opportunities such as the resolution of phylogenies, the sexing of hominid and animal remains, and the characterization of genes involved in phenotypic traits. However, to date, single-copy nuclear DNA sequences from fossils have been determined only from bones and teeth of woolly mammoths preserved in the permafrost [1]. Since the best preserved ancient nucleic acids tend to stem from cold environments [2 and 3], this has led to the assumption that nuclear DNA would be retrievable only from frozen remains. We have previously shown that Pleistocene coprolites stemming from the extinct Shasta sloth (Nothrotheriops shastensis, Megatheriidae) contain mitochondrial (mt) DNA from the animal that produced them as well as chloroplast (cp) DNA from the ingested plants [4]. Recent attempts to resolve the phylogeny of two families of extinct sloths by using strictly mitochondrial DNA has been inconclusive [5]. We have prepared DNA extracts from a ground sloth coprolite from Gypsum Cave, Nevada, and quantitated the number of mtDNA copies for three different fragment lengths by using real-time PCR. We amplified one multicopy and three single-copy nuclear gene fragments and used the concatenated sequence to resolve the phylogeny. These results show that ancient single-copy nuclear DNA can be recovered from warm, arid climates. Thus, nuclear DNA preservation is not restricted to cold climates.

Recent inquiries have revealed a surprisingly large number (>2500) of naturally occurring antisense transcripts [1, 2, 3 and 4], but their function remains largely undiscovered. A better understanding of antisense mechanisms is clearly needed because of their potentially diverse roles in gene regulation and disease [5, 6, 7 and 8]. A well-documented case occurs in X inactivation, the mechanism by which X-linked gene expression is equalized between XX females and XY males [9]. The antisense gene Tsix [6] determines X chromosome choice and represses the noncoding silencer, Xist [10, 11 and 12]. In principle, Tsix action may involve RNA, the act of transcription, or local chromatin. Here, we create novel Tsix alleles to distinguish transcription-versus RNA-based mechanisms. When Tsix transcription is terminated before Xist (TsixTRAP), Tsix cannot block Xist upregulation, suggesting the importance of overlapping antisense transcription. To separate the act of transcription from RNA, we knocked in Tsix cDNA in the reverse orientation (TsixcDNA) to restore RNA levels in cis without concurrent transcription across Xist. However, TsixcDNA cannot complement TsixTRAP. Surprisingly, both mutations disrupt choice, indicating that this epigenetic step requires transcription. We conclude that the processed antisense RNA does not act alone and that Tsix function specifically requires antiparallel transcription through Xist. A mechanism of transcription-based feedback regulation is proposed.
The development of 5′ nuclease assays represents a significant advance in nucleic acid quantitation. This approach utilizes the 5′-3′ exonuclease activity of Thermus aquaticus (Taq) polymerase to cleave a dual-labelled probe annealed to a target sequence during amplification. The release of a fluorogenic tag from the 5′ end of the probe is proportional to the target sequence concentration (copy number), and can be measured either at endpoint (post-amplification), or in ‘real time’, where the increase in emission intensity is followed on a per-cycle basis.

After a decade of intensive use as an in vitro alternative to cloning DNA, PCR is now well established as the default method for DNA and RNA analysis. Recent developments have consolidated this position by the introduction of more robust formats, improvements in thermal cyclers and labelling and detection methods. The trend is towards increasing automation, although comparatively few diagnostic kits based on PCR are in wide use. At the same time the applications of PCR are being extended with modifications such as long, accurate PCR and arrayed oligonucleotides or expressed sequences.

Background: We previously reported that the carotid artery intima-media thickness (IMT) increased with age and that patients with type 2 diabetes mellitus (DM) had a significantly larger IMT than did age-matched nondiabetic subjects with normal glucose tolerance. Although the exact mechanism behind the increase in IMT in diabetic patients has not been determined, data obtained from in vivo and in vitro studies suggest that hyperglycemia-induced oxidative stress may lead to atherogenesis. Objective: The aim of this single-center study was to determine whether long-term oxidative stress and the carotid IMT are influenced by differences of the angiotensin-converting enzyme insertion/deletion (ACE I/D) and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) oxidase p22phox C242T genotypes. Methods: Eligible subjects were Japanese patients with type 2 DM. Polymorphism of the ACE I/D and p22phox gene was investigated using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism, respectively. The rate of an acquired mutation of mitochondrial DNA—that is, A-to-G substitution at position 3243 (mtDNA A3243G)—was determined by real-time PCR. As a marker of early atherosclerosis, the carotid artery IMT was measured using high-resolution B-mode ultrasonography. Results: A total of 262 Japanese patients (173 men, 89 women; mean [SEM] age, 58 [0.6] years [range, 18-80 years]) were recruited and enrolled for study. An ACE D-positive (DD or DI) and p22phox 242T-negative genotype (CC) was associated with a significantly higher mtDNA A3243G mutation rate than the other 3 possible genotypes (0.0219% [0.0028%] vs 0.0097% [0.0012%]; P P Conclusion: In this study, the ACE D-positive and p22phox 242T-negative genotype showed higher rates of somatic mtDNA mutation (mtDNA A3243G) and higher carotid mean and maximum IMT levels.


We have characterized the expression of six cytokine mRNAs in highly purified B cells from bovine leukemia virus (BLV)-infected cows with persistent lymphocytosis. Selected cytokine mRNAs included those encoding tumor necrosis factor (TNF), lymphotoxin-[alpha] (LT-[alpha]), transforming growth factor-[beta]1 (TGF-[beta]1), interleukin-1[beta] (IL-1[beta]), interleukin-6 (IL-6) and interleukin-10 (IL-10). Fresh B cells from cows with persistent lymphocytosis constitutively transcribed TNF, LT-[alpha] and TGF-[beta]1 mRNAs. Although IL-1[beta], IL-6 and IL-10 mRNAs
were barely detectable in fresh B cells from cows with persistent lymphocytosis, transcripts encoding these cytokines were strongly and rapidly upregulated in B cells after cell culture. Results from this study provide the first evidence that B cells infected with BLV express specific cytokine mRNAs in vivo.


http://www.sciencedirect.com/science/article/B6WDF-4CXTVR8-2/2/f4501a989f0870638749c33dfa031de1

In clinical practice, diagnosis and risk prediction are usually based on the analysis of serum or plasma proteins whereas gene expression analysis is not used on a routine basis. In order to compare the diagnostic and predictive relevance of serum protein and peripheral blood mRNA levels, we determined cytokine levels of end-stage renal failure patients undergoing hemodialysis. These patients face a high mortality mainly due to acceleration of atherosclerosis and subsequent severe vascular events. mRNA expression of the pro-inflammatory cytokine TNF[alpha] was significantly elevated in hemodialysis patients and further increased after 2 h of dialysis treatment. In contrast, gene expression of the anti-inflammatory cytokine TGF[beta] was significantly decreased. Patients who died during the observation period of 36 months had significantly increased mRNA levels of TNF[alpha] and decreased TGF[beta] mRNA expression at baseline. Survival analysis indicated that increased TNF[alpha] mRNA levels (PP<0.001) predict mortality. The corresponding cytokines in serum showed some association with disease, but serum concentrations neither changed during hemodialysis nor predicted mortality. This study shows that gene expression patterns of circulating leukocytes may present an important new diagnostic tool to predict clinical outcome in patients with inflammatory vascular diseases.


Sequence data for type I interferons (IFNs) have previously only been available for birds and eutherian ('placental') mammals, but not for the other two groups of extant mammals, the marsupials and monotremes. This has left a large gap in our knowledge of the evolutionary and functional relationships of what is a complex gene family in eutherians. In this study, a PCR-based survey of type I IFN genes from a marsupial, the tammar wallaby (Macropus eugenii), and a monotreme, the short-beaked echidna (Tachyglossus aculeatus), was conducted. Along with Southern blot and phylogenetic analysis, this revealed a large number of type I IFN genes for the wallaby, rivaling that of eutherians, but relatively few type I IFN genes in the echidna. The wallaby genes include both IFNA and IFNB orthologues, indicating that the gene duplication leading to these subtypes occurred prior to the divergence of marsupials and eutherians some 130 million years ago. Results from this study support the idea that the expansion of type I IFN gene complexity in mammals coincides with a concomitant expansion in the functionality of these molecules. For example, this expansion in complexity may have, at least partially, facilitated the evolution of viviparity in marsupials and eutherians. Other evolutionary aspects of these sequences are also discussed.

http://www.sciencedirect.com/science/article/B6WDF-4C6J6YT-S4/2/a08bca4d3a8da566f10bfce1bdde4964

Recombinant tumor necrosis factor alpha (TNF-[alpha]) administration significantly delayed the development of lupuslike nephritis in the New Zealand black x New Zealand white (NZB x NZW)F1 and to a lesser extent in the MRL-lpr/lpr model systems. TNF-[alpha] treatment was effective when treatment was initiated at 2, 3, or 4 months of age but was ineffective if initiated as late as 6.5 months of age. Treatment of (NZB x NZW)F1 mice for 3 months was more effective than treatment continued for 6 months. Anti-TNF-[alpha] antibodies did not develop in these mice. Flow microfluorometry analysis showed no major effects on B, T, or monocyte cell population in cells from the peritoneum, spleen, lymph node, and thymus. A decrease in class II la expression on macrophages in the peritoneum of TNF-[alpha]-treated mice was noticed. A correlation between the level of TNF-[alpha] inducibility in vitro and the effect of TNF-[alpha] administration in vivo could be shown. Although a limited polymorphism could be shown by restriction fragment length polymorphism, using an amplified (AC)n microsatellite located in the 5' regulatory region of TNF-[alpha], a much more extensive interallelic polymorphism was found. The AC microsatellite allele found in NZW mice was unique and different from other lupus strains and nonautoimmune strains. These results have possible implications to the pathogenesis of systemic lupus erythematosus.


http://www.sciencedirect.com/science/article/B6WDF-4CXTVR8-4/2/73902d8513acd22224ac6e5fb5d766a2

Single nucleotide polymorphisms (SNPs), particularly those within regulatory regions of genes that code for cytokines often impact expression levels and can be disease modifiers. Investigating associations between cytokine genotype and disease outcome provides valuable insight into disease etiology and potential therapeutic intervention. Traditionally, genotyping for cytokine SNPs has been conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a low throughput technique not amenable for use in large-scale cytokine SNP association studies. Recently, Taqman(R) real-time PCR chemistry has been adapted for use in allelic discrimination assays. The present study validated the accuracy and utility of real-time PCR technology for a number of commonly studied cytokine polymorphisms known to influence chronic inflammatory diseases. We show that this technique is amenable to high-throughput genotyping and overcomes many of the problematic features associated with PCR-RFLP including post-PCR manipulation, non-standardized assay conditions, manual allelic identification and false allelic identification due to incomplete enzyme digestion. The real-time PCR assays are highly accurate with an error rate in the present study of <1% and concordance rate with PCR-RFLP genotyping of 99.4%. The public databases of cytokine polymorphisms and validated genotyping assays highlighted in the present study will greatly benefit this important field of research.

Knerr, K., R. Futh, et al. "Chronic inflammation and hemodialysis reduce immune competence of peripheral blood leukocytes in end-stage renal failure patients." Cytokine In Press, Corrected Proof http://www.sciencedirect.com/science/article/B6WDF-4FH0W7K-4/2/765a75a9776ed2a5e44cc9a7d6a568ea
Immunoincompetence is a profound problem in end-stage renal failure patients undergoing hemodialysis, and chronic inflammation with altered serum levels of inflammation markers has been reported. Gene expression patterns have had little relevance for leukocyte research so far because of limitations in transcript levels and stability. Using a new stimulation system we induced the expression of immune-relevant transcripts in whole blood preparations ex vivo and stabilized transcript levels by preventing RNA degradation and uncontrolled gene induction. Using quantitative real-time PCR we could show that basal TGF-[beta] mRNA expression is about 2-fold decreased in end-stage renal failure patients, while expression of TNF-[alpha] becomes 2-fold increased, further doubling during hemodialysis. By short term stimulation with phytohemagglutinin (PHA) for 2 h we tested for the immune competence of peripheral blood leukocytes and demonstrated that hemodialysis decreases TNF-[alpha]-mediated immune responsiveness more than 3-fold. This study shows by induction and stabilization of immune-relevant transcripts that chronic inflammation and hemodialysis are crucial factors for disturbed immune competence of end-stage renal failure patients.


Background/AimsIn hepatitis C virus infection an inappropriate ratio of pro-inflammatory and anti-inflammatory cytokines may either determine different outcomes of the infection or affect the benefit of antiviral treatment. Given that polymorphisms in regulatory regions of cytokine genes influence cytokine production, we determined frequency of polymorphisms of IL-10, IFN[gamma], and TNF[alpha] genes in HCV-infected patients and healthy controls, and investigated their association with either ongoing or cleared HCV infection, or with response to treatment.

MethodsGenomic DNA from 270 patients and 145 controls sharing the same ethnic background was studied by polymerase chain reaction, restriction enzyme digestion, direct sequencing, and microsatellite analysis.

ResultsThe IL-10 ATA haplotype was more frequent in patients with spontaneous HCV RNA clearance (36.0%) than in patients with persistent infection (23%) (p=0.009, p CORRECTED = 0.036). Neither TNF -308 and -238 polymorphisms nor IFN[gamma] alleles variability were associated with different HCV outcome. However, the combination of ATA homozygous state and IFN[gamma] 119 allele was more frequent in patients with spontaneous HCV clearance than in patients with ongoing disease (p=0.012; p CORRECTED = 0.048). We could not confirm the reported effect of genetic influence on the response to treatment.

ConclusionsOur findings indicate that heterogeneity in the promoter region of the IL-10 gene has a role in determining a spontaneous favourable outcome of HCV infection.


Molecular cloning of canine interleukin-8 (IL-8) was performed to establish a basis for its investigation in the canine immune system. From a cDNA pool constructed from LPS-stimulated popliteal lymph node cells, canine IL-8 cDNA covering the whole coding region was amplified by polymerase chain reaction. The nucleotide sequence of a canine IL-8 clone, designated pclL-8#38, was highly similar to those of human, rabbit and porcine IL-8, and comprised 353 bp with an open reading frame that encoded 101 amino acids. Analysis of the deduced amino acid sequence of insert DNA in pclL-8#38 showed 76.5, 80.2, and 87.0% similarities with human,
rabbit and porcine IL-8 proteins, respectively. Insert DNA of pcIL-8#38 was transferred to a mammalian expression vector, pcDL-SR[alpha]296, and transfected into Cos7 cells. The supernatant of the transfectant had neutrophil chemotactic activity when it was examined by the neutrophil migration assay, suggesting that our cloned cDNA was biologically active. The cloned canine IL-8 cDNA will be useful for canine inflammatory disease and comparative immunology research.


http://www.sciencedirect.com/science/article/B6WDF-4CP68M7-4/2/8607489d33c1768c0b8ad812e40474d8

Aotus spp. monkeys are considered the ideal model for studying the progress of malarial infection and the immune response it elicits. We describe the use of a recently developed technique, real-time quantitative RT-PCR, to quantify several Aotus monkey cytokine mRNAs involved in Th1/Th2 responses (IL-4, IL-10, TNF-[beta] and IFN-[gamma]). Specific primers were designed for each cytokine and standard curves were constructed using serial dilutions of pDNA containing each target sequence. Results were normalized to GAPDH housekeeping gene expression levels. Standard curves showed high correlation coefficients and were linear over a wide range of copy numbers. Quantification of Aotus samples showed little intra- and inter-experiment variation, thus, the technique has proven to be highly reproducible and sensitive allowing us to detect as little as 25 copies/µl of target DNA. This technique will allow studying Th1 and Th2 cytokine patterns elicited in response to infection for prospectively evaluating the efficacy of malarial vaccines.


http://www.sciencedirect.com/science/article/B6WDF-4CF57-F-4G/2/ff10a5e47ecf632fee69eb65392500d0

Kit ligand, or stem cell factor, is a recently identified growth factor, which binds to and activates the c-kit proto-oncogene, and which has been shown to act synergistically with other haematopoietic growth factors in the bone marrow. We have previously shown that several isoforms of kit ligand, which arise due to alternative splicing, are expressed in human placenta. In order to elucidate the role of c-kit and its ligand during human placental development we have investigated the expression of c-kit and kit ligand in human first trimester and term placenta as well as in pregnant and non-pregnant endometrium, by immunocytochemistry and flow cytometric analysis. In non-pregnant endometrium no expression of kit ligand was seen. By contrast, in first trimester decidua, kit ligand was strongly expressed by the arterial media of maternal blood vessels. Kit ligand was also expressed throughout pregnancy by invasive fetal extravillous trophoblast, and by fetal fibroblasts within the placental villi. c-kit was found to be expressed on Hofbauer cells within the chorionic villi, and by decidual macrophages at all stages in pregnancy. c-kit was also detected on the small CD56dim subset of uterine large granular lymphocytes which form the major leukocyte population in human first trimester decidua. Our results suggest that kit ligand may be involved in the regulation of fetal macrophages, and in particular in signalling between invading extravillous trophoblast which expresses kit ligand, and maternal leukocytes bearing the c-kit receptor.

http://www.sciencedirect.com/science/article/B6WDF-49M0KT1-2/2/31a33f1a1a5d527995b053d8164f6ed5

The goal of this research was to determine whether differential pulmonary IL-12 gene expression controls susceptibility to Sendai virus-induced chronic airway inflammation and fibrosis in inbred rat strains. Sendai virus-resistant F344 rats and susceptible BN rats were studied from 1 to 14 days following virus inoculation. F344 rats had 3.4-fold higher IL-12 mRNA levels detected by real-time PCR in lung than BN rats as early as two days following inoculation. This increase in mRNA was associated at two days with increased total IL-12 protein and with a 2-fold increase in numbers of bronchiolar, OX-6-positive dendritic cells and an increased number of IL-12 p40-positive, bronchiolar macrophages and dendritic cells (pp<0.05). The results demonstrate that there is differential pulmonary IL-12 gene expression between virus-susceptible and resistant rat strains and that IL-12 treatment can provide significant protection from virus-induced chronic airway inflammation and remodeling during early life.


http://www.sciencedirect.com/science/article/B6WDF-49505VD-3/2/4f7d2f594558a5777f1c340fbe5da7a56

The chemokine, mob-1, is involved in inflammatory and immune responses and may be an important mediator of the inflammatory response in the liver. Here, we investigated the upstream signal pathways that could be involved in the regulation of mob-1 expression. We have found that in primary rat hepatocytes the isolation and subsequent culture of these cells induced mob-1 expression. A similar induction of mob-1 mRNA was observed when the hepatocytes were stimulated with interferon-[gamma] (IFN-[gamma]). When hepatocytes were stimulated with IFN-[gamma] or cytokine mixture (IFN-[gamma], interleukin-1[beta] and tumour necrosis factor-[alpha]), c-Jun N-terminal kinase (JNK), p38 and extracellular-regulated kinase (ERK) were phosphorylated, suggesting an involvement of the mitogen-activated protein kinases (MAPK) in the induction of mob-1 expression. The p38 kinase inhibitor, SB 203580, and the NF-[kappa]B inhibitor, MG-132, inhibited the induction of mob-1 mRNA and the effects were not additive. These results demonstrate that in primary rat hepatocytes the transient induction of mob-1 expression was regulated by p38 kinase and NF-[kappa]B through a common regulatory pathway.


http://www.sciencedirect.com/science/article/B6WDF-4F02GVT-3/2/2df4efaa4b5d97c92c740397f5b92444

The aim of this study was to evaluate whether there was any correlation between Helicobacter pylori-associated diseases and (1) H. pylori virulence genes or (2) IL-1B, IL-1RN, IFN-G, TNF-A, IL-10 genetic polymorphisms. Patients with non-cardia gastric cancer (NCGC, n = 129) or benign gastroduodenal diseases (n = 792) were studied. IL-1RN intron 2 VNTR polymorphism (PCR), IL-1B -31 C/T (RFLP), the SNPs of IFN-G (+874 A/T), TNF-A (-1031 C/T, -857 C/T, -376 A/G, -308 A/G, -238 A/G), IL-10 (-1082 A/G, -819 C/T, -592 A/C) (Taqman chemistry) were studied. cagA,
s1 and m1 vacA, were PCR amplified. Duodenal ulcer was more frequent in TNF-A -857 TT and in IL-1RN 1,2 subjects. TNF-A -857 TT genotype was also correlated with gastric ulcer. IL-10 -819 TT genotype was associated with intestinal metaplasia and NCGC. Antral inflammation was associated with TNF-A -1031 TT, while corpus activity with IL-10 -819 CC. H. pylori infection was associated with TNF-A -308 AG genotype, while IFN-G +874 AA genotype was associated with cagA. In conclusion, among host genetic factors contributing to H. pylori disease outcome, IFN-G +874 AA genotype favors cagA positive infections, TNF-A -857 TT duodenal ulcer while IL-10 -819 TT intestinal metaplasia and NCGC.

Deep Sea Research Part II: Topical Studies in Oceanography (1)


http://www.sciencedirect.com/science/article/B6VGC-3Y0RMGY-C/2/3e7916c1e45f22b3a75ee8271b9027a6

Horizontal and vertical variations in bacterial community composition were examined in samples collected during two Joint Global Ocean Flux Study (JGOFS) Arabian Sea cruises in 1995. The cruises, 11 months apart, took place during two consecutive NE Monsoon periods (January and December). Bacteria were harvested by filtration from samples collected in the mixed layer, mid-water, and deep sea at stations across the study area. Total bacterial community genomic DNA was analyzed by PCR amplification of 16S rRNA gene fragments, followed by denaturing gradient gel electrophoresis (DGGE). In total, 20 DGGE bands reflecting unique or varying phylotypes were excised, cloned and sequenced. Amplicons were dominated by bacterial groups commonly found in oceanic waters (e.g., the SAR11 cluster of [alpha]-Proteobacteria and cyanobacteria), but surprisingly none of the sequenced amplicons were related to [gamma]-Proteobacteria or to members of the Cytophaga-Flavobacter-Bacteroides phylum. Amplicons related to magnetotactic bacteria were found for the first time in pelagic oceanic waters. The DGGE banding patterns revealed a dominance of [ap]15 distinguishable amplicons in all samples. In the mixed layer the bacterial community was dominated by the same [ap]15 phylotypes at all stations, but unique phylotypes were found with increasing depth. Except for cyanobacteria, comparison of the bacterial community composition in surface waters from January and December 1995 showed only minor differences, despite significant differences in environmental parameters. These data suggest a horizontal homogeneity and some degree of seasonal predictability of bacterial community composition in the Arabian Sea.

Desalination (1)

Parallel experiments using a blend of surface waters were conducted to evaluate differential fouling rates among reverse osmosis (RO) membranes when operated under pilot- vs. full-scale conditions. Testing was conducted using a 230 L/min conventional (rapid mix/flocculation/sedimentation/filtration) package plant (CPP) and a 2,000 ML/d fullscale treatment plant (FTP) as pretreatment to separate RO membrane test units. Coagulation consisted of 10 mg/L alum (as Al2(SO4)3-14H2O) and 2.0 mg/L cationic polymer. A2.5-3.0 mg/L free-chlorine residual was maintained at the filter effluent and converted to chloramines through ammonium sulfate addition (3:1 chlorine-to-ammonia w/w ratio). Membrane performance was based on normalized flux and salt rejection data. Membrane surface analyses included scanning electron microscopy, energy-dispersive spectroscopy, and attenuated total reflectance Fourier transform infrared spectroscopy. Microbial activity and community analyses were conducted through (a) fluorescence staining with 4',6'-diamidino-2-phenylindole, (b) polymerase-chain reaction amplification of isolated bacterial DNA, and (c) microscopic taxonomic identification. Results indicated that the RO membrane fed by the CPP fouled at least three times faster than the RO membrane fed by the FTP. The differential fouling between the two process streams was determined to be from lack of maintenance in the CPP influent piping that led to the establishment of biological communities consisting of algae, microbes, and, potentially, freshwater clams. These communities produced low levels of natural polymers, which when presented to the polyamide RO membrane surface, resulted in rapid fouling.

Development (12)


In animal development, digestive tissues emerge from different positions of the endoderm as a result of patterning signals from overlying mesoderm. Although embryonic tissue movement during gastrulation generates an initial positional relationship between the endoderm and mesoderm, the role of subsequent endoderm movement against the mesoderm in patterning is unknown. At embryonic day 8.5 in the mouse, proliferation of cells at the leading edge of ventral-lateral endoderm, where the liver and ventral pancreas emerge, helps close off the foregut. During this time, the endoderm grows adjacent to and beyond the cardiogenic mesoderm, an inducer of the liver program and an inhibitor of the pancreas program. The homeobox gene Hex is expressed in this endoderm cell domain and in the liver and ventral pancreas buds, after organogenesis. We have found that in Hex-/- embryos, there is a complete failure in ventral pancreatic specification, while the liver program is still induced. However, when Hex-null ventral endoderm is isolated prior to its interaction with cardiogenic mesoderm and is cultured in vitro, it activates early pancreas genes. We found that Hex controls the proliferation rate, and thus the positioning, of the leading edge of endoderm cells that grow beyond the cardiogenic mesoderm, during gut tube closure. Thus, Hex-controlled positioning of endoderm cells beyond cardiogenic mesoderm dictates ventral pancreas specification. Other endodermal transcription factors may also function morphogenetically rather than by directly regulating tissue-specific programs.

http://dev.biologists.org/cgi/content/abstract/129/21/4879

In the stretch-reflex system, proprioceptive sensory neurons make selective synaptic connections with different subsets of motoneurons, according to the peripheral muscles they supply. To examine the molecular mechanisms that may influence the selection of these synaptic targets, we constructed single-cell cDNA libraries from sensory neurons that innervate antagonist muscles. Differential screening of these libraries identified a transcription regulatory co-factor of the LIM homeodomain proteins, the LIM domain only 4 protein Lmo4, expressed in most adductor but few sartorius sensory neurons. Differential patterns of Lmo4 expression were also seen in sensory neurons supplying three other muscles. A subset of motoneurons also expresses Lmo4 but the pattern of expression is not specific for motor pools. Differential expression of Lmo4 occurs early, as neurons develop their characteristic LIM homeodomain protein expression patterns. Moreover, ablation of limb buds does not block Lmo4 expression, suggesting that an intrinsic program controls the early differential expression of Lmo4. LIM homeodomain proteins are known to regulate several aspects of sensory and motor neuronal development. Our results suggest that Lmo4 may participate in this differentiation by regulating the transcriptional activity of LIM homeodomain proteins.


http://dev.biologists.org/cgi/content/abstract/131/21/5309

Previous work has revealed that proteins that bind to bone morphogenetic proteins (BMPs) and inhibit their signalling have a crucial role in the spatial and temporal regulation of cell differentiation and cell migration by BMPs. We have identified a chick homologue of crossveinless 2, a Drosophila gene that was identified in genetic studies as a promoter of BMP-like signalling. Chick Cv-2 has a conserved structure of five cysteine-rich repeats similar to those found in several BMP antagonists, and a C-terminal Von Willebrand type D domain. Cv-2 is expressed in the chick embryo in a number of tissues at sites at which elevated BMP signalling is required. One such site of expression is premigratory neural crest, in which at trunk levels threshold levels of BMP activity are required to initiate cell migration. We show that, when overexpressed, Cv-2 can weakly antagonise BMP4 activity in Xenopus embryos, but that in other in vitro assays Cv-2 can increase the activity of co-expressed BMP4. Furthermore, we find that increased expression of Cv-2 causes premature onset of trunk neural crest cell migration in the chick embryo, indicative of Cv-2 acting to promote BMP activity at an endogenous site of expression. We therefore propose that BMP signalling is modulated both by antagonists and by Cv-2 that acts to elevate BMP activity.


http://dev.biologists.org/cgi/content/abstract/131/9/2073

Plexins are functional receptors for Semaphorin axon guidance cues. Previous studies have established that some Plexins directly bind RACGTP and RHO. Recent work in C. elegans showed that semaphorin 1 (smp-1 and smp-2) and plexin 1 (plx-1) are required to prevent
anterior displacement of the ray 1 cells in the male tail (Fujii et al., 2002; Ginzburg et al., 2002). We show genetically that plx-1 is part of the same functional pathway as smp-1 and smp-2 for male ray positioning. RAC GTPase genes mig-2 and ced-10 probably function redundantly, whereas unc-73, which encodes a GEF for both of these GTPases, is required cell autonomously for preventing anterior displacement of ray 1 cells. RNAi analysis indicates that rho-1-encoded RHO GTPase, plus let-502 and K08B12.5-encoded RHO-kinases, are also required to prevent anterior displacement of ray 1 cells, suggesting that different kinds of RHO-family GTPases act similarly in ray 1 positioning. At low doses of wild-type mig-2 and ced-10, the Semaphorin 1 proteins no longer act through PLX-1 to prevent anterior displacements of ray 1, but have the opposite effect, acting through PLX-1 to mediate anterior displacements of ray 1. These results suggest that Plexin 1 senses levels of distinct RHO and RAC GTPases. At normal levels of RHO and RAC, Semaphorin 1 proteins and PLX-1 prevent a forward displacement of ray 1 cells, whereas at low levels of cycling RAC, Semaphorin 1 proteins and PLX-1 actively mediate their anterior displacement. Endogenously and ectopically expressed SMP-1 and SMP-2 suggest that the hook, a major source of Semaphorin 1 proteins in the male tail, normally attracts PLX-1-expressing ray 1 cells.


http://dev.biologists.org/cgi/content/abstract/129/9/2065

The semaphorin family comprises secreted and transmembrane proteins involved in axon guidance and cell migration. We have isolated and characterized deletion mutants of C. elegans semaphorin 1a (Ce-sema-1a or smp-1) and semaphorin 1b (Ce-sema-1b or smp-2) genes. Both mutants exhibit defects in epidermal functions. For example, the R1.a-derived ray precursor cells frequently fail to change anterior/posterior positions completely relative to their sister tail lateral epidermal precursor cell R1.p, causing ray 1 to be formed anterior to its normal position next to ray 2. The ray cells, which normally separate from the lateral tail seam cell (SET) at the end of L4 stage, remains connected to the SET cell even in adult mutant males. The ray 1 defects are partially penetrant in each single Ce-sema-1 mutant at 20(degrees)C, but are greatly enhanced in Ce-sema-1 double mutants, suggesting that Ce-Sema-1a and Ce-Sema-1b function in parallel to regulate ray 1 position. Both mutants also have defects in other aspects of epidermal functions, including head and tail epidermal morphogenesis and touch cell axon migration, whereas, smp-1 mutants alone have defects in defecation and brood size. A feature of smp-1 mutants that is shared with mutants of mab-20 (which encodes Sema-2a) is the abnormal perdurance of contacts between epidermal cells.


http://dev.biologists.org/cgi/content/abstract/130/8/1645

Signaling by the hedgehog (hh)-class gene pathway is essential for embryogenesis in organisms ranging from Drosophila to human. We have isolated a hh homolog (Hro-hh) from a lophotrochozoan species, the glossiphoniid leech, Helobdella robusta, and examined its expression by reverse transcription polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization. The peak of Hro-hh expression occurs during organogenesis (stages 10-11). No patterned expression was detected within the segmented portion of the germinal plate during the early stages of segmentation. In stage 10-11 embryos, Hro-hh is expressed in body wall, foregut, anterior and posterior midgut, reproductive organs and in a subset of ganglionic neurons.
Evidence that Hro-hh regulates gut formation was obtained using the steroidal alkaloid
cyclopamine, which specifically blocks HH signaling. Cyclopamine induced malformation of both
foregut and anterior midgut in Helobdella embryos, and no morphologically recognizable gonads
were seen. In contrast, no gross abnormalities were observed in the posterior midgut. Segmental
ectoderm developed normally, as did body wall musculature and some other mesodermal
derivatives, but the mesenchymal cells that normally come to fill most of the coelomic cavities
failed to develop. Taken with data from Drosophila and vertebrates, our data suggest that the role
of hh-class genes in gut formation and/or neural differentiation is ancestral to the bilaterians,
whereas their role in segmentation evolved secondarily within the Ecdysozoa.

antagonistically to Orb in the oskar mRNA localization and translation pathway.” Development
129(1): 197-209.

http://dev.biologists.org/cgi/content/abstract/129/1/197

Subcellular localization of mRNAs within the Drosophila oocyte is an essential step in body
patterning. Yps, a Drosophila Y-box protein, is a component of an ovarian ribonucleoprotein
complex that also contains Exu, a protein that plays an essential role in mRNA localization. Y-box
proteins are known translational regulators, suggesting that this complex might regulate
translation as well as mRNA localization. Here we examine the role of the yps gene in these
events. We show that yps interacts genetically with orb, a positive regulator of oskar mRNA
localization and translation. The nature of the genetic interaction indicates that yps acts
antagonistically to orb. We demonstrate that Orb protein is physically associated with both the
Yps and Exu proteins, and that this interaction is mediated by RNA. We propose a model wherein
Yps and Orb bind competitively to oskar mRNA with opposite effects on translation and RNA
localization.

regulates development of basal pattern elements of the Arabidopsis gynoecium.” Development
131(15): 3737-3749.

http://dev.biologists.org/cgi/content/abstract/131/15/3737

Flowers of the parthenocarpic knuckles mutant are conditionally male sterile and contain ectopic
stamens and carpels that originate from placental tissue within developing gynoecia. The
mutation was mapped to a 123 Kb interval on chromosome 5 using molecular markers. All
aspects of the knuckles phenotype could be complemented by a genomic fragment from the
region which contained the annotated MAC12.2 gene. A guanine to adenine transition within a
predicted C2H2 zinc finger-encoding region of MAC12.2 causes the second of two critical zinc-
binding cysteine residues to be replaced by a tyrosine. Transgenic plants in which translational
fusions of the GUS reporter to KNUCKLES were driven by the presumptive KNUCKLES promoter
indicate that the gene is expressed first in developing carpel primordia, and later in stamens and
ovules of flower buds. In situ hybridization experiments showed a broader pattern of transcript
localization, suggesting that post-transcriptional regulatory mechanisms may limit KNUCKLES
protein accumulation and localization. Based on genetic evidence and the presence of a carboxy-
terminal motif demonstrated by others to function as an active repression domain, we propose
that KNUCKLES might function as a transcriptional repressor of cellular proliferation that
regulates floral determinacy and relative size of basal pattern elements along the proximo-distal
axis of the developing Arabidopsis gynoecium.

http://dev.biologists.org/cgi/content/abstract/130/8/1565

Sonic hedgehog is involved in eye field separation along the proximodistal axis. We show that Hh signalling continues to be important in defining aspects of the proximodistal axis as the optic vesicle and optic cup mature. We show that two other Hedgehog proteins, Banded hedgehog and Cephalic hedgehog, related to the mouse Indian hedgehog and Desert hedgehog, respectively, are strongly expressed in the central retinal pigment epithelium but excluded from the peripheral pigment epithelium surrounding the ciliary marginal zone. By contrast, downstream components of the Hedgehog signalling pathway, Gli2, Gli3 and X-Smoothened, are expressed in this narrow peripheral epithelium. We show that this zone contains cells that are in the proliferative state. This equivalent region in the adult mammalian eye, the pigmented ciliary epithelium, has been identified as a zone in which retinal stem cells reside. These data, combined with double labelling and the use of other retinal pigment epithelium markers, show that the retinal pigment epithelium of tadpole embryos has a molecularly distinct peripheral to central axis. In addition, Gli2, Gli3 and X-Smoothened are also expressed in the neural retina, in the most peripheral region of the ciliary marginal zone, where retinal stem cells are found in Xenopus, suggesting that they are good markers for retinal stem cells. To test the role of the Hedgehog pathway at different stages of retinogenesis, we activated the pathway by injecting a dominant-negative form of PKA or blocking it by treating embryos with cyclopamine. Embryos injected or treated at early stages display clear proximodistal defects in the retina. Interestingly, the main phenotype of embryos treated with cyclopamine at late stages is a severe defect in RPE differentiation. This study thus provides new insights into the role of Hedgehog signalling in the formation of the proximodistal axis of the eye and the differentiation of retinal pigment epithelium.


http://dev.biologists.org/cgi/content/abstract/131/19/4775

Sympathetic neurons are specified during their development from neural crest precursors by a network of crossregulatory transcription factors, which includes Mash1, Phox2b, Hand2 and Phox2a. Here, we have studied the function of Gata2 and Gata3 zinc-finger transcription factors in autonomic neuron development. In the chick, Gata2 but not Gata3 is expressed in developing sympathetic precursor cells. Gata2 expression starts after Mash1, Phox2b, Hand2 and Phox2a expression, but before the onset of the noradrenergic marker genes Th and Dbh, and is maintained throughout development. Gata2 expression is affected in the chick embryo by Bmp gain- and loss-of-function experiments, and by overexpression of Phox2b, Phox2a, Hand2 and Mash1. Together with the lack of Gata2/3 expression in Phox2b knockout mice, these results characterize Gata2 as member of the Bmp-induced cluster of transcription factors. Loss-of-function experiments resulted in a strong reduction in the size of the sympathetic chain and in decreased Th expression. Ectopic expression of Gata2 in chick neural crest precursors elicited the generation of neurons with a non-autonomic, Th-negative phenotype. This implies a function for Gata factors in autonomic neuron differentiation, which, however, depends on co-regulators present in the sympathetic lineage. The present data establish Gata2 and Gata3 in the chick and mouse, respectively, as essential members of the transcription factor network controlling sympathetic neuron development.

http://dev.biologists.org/cgi/content/abstract/131/12/2921

Hemps, a novel epidermal growth factor (EGF)-like protein, is expressed during larval development and early metamorphosis in the ascidian Herdmania curvata and plays a direct role in triggering metamorphosis. In order to identify downstream genes in the Hemps pathway we used a gene expression profiling approach, in which we compared post-larvae undergoing normal metamorphosis with larval metamorphosis blocked with an anti-Hemps antibody. Molecular profiling revealed that there are dynamic changes in gene expression within the first 30 minutes of normal metamorphosis with a significant portion of the genome (approximately 49%) being activated or repressed. A more detailed analysis of the expression of 15 of these differentially expressed genes through embryogenesis, larval development and metamorphosis revealed that while there is a diversity of temporal expression patterns, a number of genes are transiently expressed during larval development and metamorphosis. These and other differentially expressed genes were localised to a range of specific cell and tissue types in Herdmania larvae and post-larvae. The expression of approximately 24% of the genes that were differentially expressed during early metamorphosis was affected in larvae treated with the anti-Hemps antibody. Knockdown of Hemps activity affected the expression of a range of genes within 30 minutes of induction, suggesting that the Hemps pathway directly regulates early response genes at metamorphosis. In most cases, it appears that the Hemps pathway contributes to the modulation of gene expression, rather than initial gene activation or repression. A total of 151 genes that displayed the greatest alterations in expression in response to anti-Hemps antibody were sequenced. These genes were implicated in a range of developmental and physiological roles, including innate immunity, signal transduction and in the regulation of gene transcription. These results suggest that there is significant gene activity during the very early stages of H. curvata metamorphosis and that the Hemps pathway plays a key role in regulating the expression of many of these genes.


http://dev.biologists.org/cgi/content/abstract/129/12/2891

In order to assess the in vivo function of integrins containing the (beta)8 subunit, we have generated integrin (beta)8-deficient mice. Ablation of (beta)8 results in embryonic or perinatal lethality with profound defects in vascular development. Sixty-five percent of integrin (beta)8-deficient embryos die at midgestation, with evidence of insufficient vascularization of the placenta and yolk sac. The remaining 35% die shortly after birth with extensive intracerebral hemorrhage. Examination of brain tissue from integrin (beta)8-deficient embryos reveals abnormal vascular morphogenesis resulting in distended and leaky capillary vessels, as well as aberrant brain capillary patterning. In addition, endothelial cell hyperplasia is found in these mutant brains. Expression studies show that integrin (beta)8 transcripts are localized in endodermal cells surrounding endothelium in the yolk sac and in periventricular cells of the neuroepithelium in the brain. We propose that integrin (beta)8 is required for vascular morphogenesis by providing proper cues for capillary growth in both yolk sac and embryonic brain. This study thus identifies a molecule crucial for vascular patterning in embryonic yolk sac and brain.
We have cloned and characterized a cDNA encoding Cg-tal in the Pacific oyster Crassostrea gigas. The isolated cDNA encodes a 219 amino acids protein that contains the basic helix-loop-helix (bHLH) domain homologous to that of vertebrate and invertebrate Tal1/SCL. Phylogenetic analyses sustained that Cg-Tal belongs to this family of bHLH transcription factors. Northern blot analysis of Cg-tal mRNA expression in adult oyster tissues indicated that Cg-ta1 was specifically expressed in hemocytes, in a constitutive manner. In vertebrates, activation of Tal1/SCL expression is essential for the initiation of hematopoiesis and the formation of hematopoietic stem cells. Considering Tal1/SCL function in vertebrates, Cg-Tal is likely to constitute a promising tool for studying hematopoiesis in oyster.


That the plasma concentration of certain divalent cations change during an inflammatory insult provides a major host defense response in vertebrate animals. This study was designed to investigate the involvement of iron sequestration in invertebrate immune responses. A ferritin molecule was cloned from an echinoderm coelomocyte cDNA library. The amino acid sequence showed sequence homology with vertebrate ferritin. The cDNA contained a conserved iron responsive element sequence. Studies showed that stimulated coelomocytes released iron into in vitro culture supernatants. The amount of iron in the supernatants decreased over time when the amoebocytes were stimulated with LPS or PMA. Coelomocytes increased expression of ferritin mRNA after stimulation. In vertebrates, cytokines can cause changes in iron levels in macrophages. Similarly, echinoderm macrokines produced decreases in iron levels in coelomocyte supernatant fluids. These results suggest that echinoderm ferritin is an acute phase protein and suggest that sequestration of iron is an ancient host defense response in animals.


Natural resistance associated macrophage protein 1 (Nramp1) affects the ability of macrophages to kill pathogens. We cloned Nramp cDNA of channel catfish to identify potential molecular markers for disease resistance. Three different Nramp transcripts were identified: NrampCa-2912
nucleotides (nt), NrampCb-3245 nt, and NrampCc-3721 nt. At the 5' end, the transcripts have a common 2263 nt sequence containing the open reading frame. The differences are in the 3' untranslated region resulting from alternative splicing and polyadenylation. NrampCc is the predominant form expressed. The deduced 550 amino acid sequence of the channel catfish Nramp (NrampC) has high homology to Nramp from other vertebrates and a predicted conserved structure. The NrampC contains the 12 transmembrane domains, and the consensus transport motif. Post-transcriptional processing is also conserved. Phylogenetic analysis grouped NrampC with other fishNramps and closer to Nramp2 than to Nramp1 of mammals. However, the catfish transcript does not contain an iron-responsive regulatory-protein binding site, a characteristic of Nramp2, and, like Nramp1, NrampC expression is induced in macrophage-rich tissues after exposure to lipopolysaccharide and in a macrophage cell line when stimulated. Thus NrampC is structurally closer to mammalian Nramp2 but may function similar to Nramp1.


http://www.sciencedirect.com/science/article/B6T5X-41V2PJW-4/2/66ec478a31dfe42eb2084f7dede8ea6a

Low molecular weight antimicrobial peptides are an important component of the innate immune system in animals, yet they have not been examined widely in fish. Of particular interest is their expression during development and in response to environmental conditions and disease. Here, we report the isolation of four genomic sequences encoding putative antimicrobial peptides from the winter flounder, Pleuronectes americanus (Walbaum), as well as reverse transcription-PCR products from two tissues that form the first defensive barrier to microbes -- skin and intestine. Alignment of the predicted polypeptide sequences shows a conserved hydrophobic signal peptide of 22 amino acids followed by 25 amino acids that are identical (WF2) or homologous to the amino acid sequence of pleurocidin, followed by a conserved acidic portion. Southern hybridisation analysis indicates that related peptides are encoded in the genomes of other flatfish species. Northern and RT-PCR analyses of RNA from multiple tissues show that two of the pleurocidin genes are expressed predominantly in the skin whereas two other genes are expressed mainly in the intestine. RT-PCR assays of total RNA from larvae of different ages provide the first evidence of developmental expression of antimicrobial peptides in fish and indicate that the pleurocidin gene is first expressed at 13 days post-hatch in winter flounder.


http://www.sciencedirect.com/science/article/B6T5X-46SNYY5-1/2/2bfc66c364686b4d4daf598b7cd27171

In the course of suppression subtractive hybridisation between sodium alginate-induced peritoneal cells (SA-PC) and normal head kidney cDNAs in common carp (Cyprinus carpio), a cytokine-like cDNA clone was found. The clone, named M17, contains a 1600 bp nucleotide sequence that encodes a 215 amino acid putative protein that would have a pI of 9.01 and would include a 33 amino acid signal peptide. The 3' untranslated region has seven ATTTA mRNA destabilising motifs that are common in cytokines and oncogenes. In a BLASTP search, M17 was most similar to chicken ciliary neurotrophic factor (CNTF) with 25% amino acid identity, followed by mammalian CNTF, cardiotrophin-1 and leukemia inhibitory factor (LIF) all of which belong to the IL-6 subfamily. However, M17 has some differences with CNTF in that CNTF has no signal
sequence, the gene organisation of M17 is three exons and two introns, whereas that of CNTF is two exons and one intron, M17 has seven cysteines while CNTF has one cysteine, and M17 mRNA is detected in peripheral blood leukocytes as well as brain, whereas CNTF is expressed only in the nervous system. Compared to other members in the IL-6 subfamily cytokines, M17’s cysteine positions and gene organisation are similar to those of oncostatin M and LIF, although amino acid identities are only 15-17%. Southern hybridisation suggested that M17 is a single copy gene. SA-PC showed significantly higher M17 mRNA levels than normal head kidney cells, which are considered to be a source of the SA-PC, indicating that M17 is inducible by inflammatory stimulation.


http://www.sciencedirect.com/science/article/B6T5X-40962SK-8/2/14df458749dbc6fe900e5060aff4db69

In order to characterize the Major histocompatibility complex (MHC) class II A genes of the channel catfish (Ictalurus punctatus) a cDNA library was screened and PCR was performed. Four different full-length cDNA sequences for MHC class II A genes were obtained from a clonal B cell line derived from an outbred fish. Two different genomic sequences and corresponding cDNAs were obtained from a presumably homozygous gynogenetic catfish. The A genes have five exons and four phase one introns. The first exon encodes the 5' untranslated region (UTR) and leader peptide; the second and third exons encode the [alpha]1 and [alpha]2 domains, respectively. The connecting peptide, transmembrane and cytoplasmic domains, as well as part of the 3' UTR, are encoded by the fourth exon and the rest of the 3' UTR is encoded by the fifth exon. Southern blot analyses using an exon three probe revealed two to four hybridizing fragments with considerable restriction fragment length polymorphisms evident among randomly selected outbred channel catfish. These findings are consistent with the presence of at least two functional polymorphic MHC class II A gene loci. An unusual aspect of the channel catfish MHC class II [alpha] chain is its lack of N-linked glycosylation sites.


http://www.sciencedirect.com/science/article/B6T5X-3W321NP-5/2/92a32e2cdbb06216ef0fe48ef932cae7

A full-length cDNA clone (Onmy-UA-C32) encoding a major histocompatibility complex (MHC) class I heavy chain was isolated from a rainbow trout thymus cDNA library. Onmy-UA-C32 alpha I and III extracellular domains were most similar to other salmonids (92 and 86% at the nucleotide and amino acid level) but interestingly the alpha II domain is closer to that of the carp (74 and 73%) and zebrafish (75 and 70%). In addition, Onmy-UA-C32 displays conservation of residues known to be essential for the function and structure of MHC class Ia molecules. Northern blot hybridization with alpha 2 or 2-3 domain probes of Onmy-UA-C32 detected high expression (2.6 kb) of this gene in the spleen, thymus, kidney, heart and intestine with lower levels being observed in the brain and liver. No tissues were found to be negative indicating a ubiquitous pattern of expression for Onmy-UA-C32. Onmy-UA-C32 may therefore represent a MHC class Ia gene in trout as well as providing new insights regarding the evolution of the MHC within teleost species. Copyright
Fibrinogen-related proteins (FREPs) are hypothesized to function in non-self-recognition in the snail Biomphalaria glabrata. To investigate this assumption, the expression of four members of the FREP gene family was studied using quantitative PCR at 0.5-16 days following exposure of M line and BS-90 strain B. glabrata to Echinostoma paraensei and Schistosoma mansoni. Both strains react to, but fail to eliminate E. paraensei. Only the BS-90 strain is immunologically resistant to S. mansoni. Both snail strains responded to E. paraensei with significantly elevated expression of FREP 2 and 4. Following exposure to S. mansoni, resistant BS-90 snails showed an increase in expression of FREP 2 and 4 (57-fold and 4.5-fold increase, respectively), susceptible M line snails did not display a FREP response. Expression of FREP 3 and 7 was not significantly elevated in any snail/trematode combination. These expression profiles support the hypothesis that some FREPs play a role in the anti-trematode responses in B. glabrata.


http://www.sciencedirect.com/science/article/B6T5X-449T5DD-8/2/be755ad0c506744b2e6d0a2a3f04341

Avian thrombocytes are nucleated blood cells homologous in function to mammalian platelets. In the present study, we obtained a cDNA from chicken thrombocyte polyadenylated RNA [Poly(A)+RNA], which coded for the chicken PDGF-B chain. The sequence was 1083-bp long and had an open reading frame (ORF) of 753-bp. At the amino acid level, the predicted mature protein showed 69% homology with the processed coding region of human PDGF-B. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis showed that PDGF-B mRNA was
expressed at high levels in thrombocytes and in the lung. The expression of PDGF-B chain mRNA in thrombocytes reached its maximum level 12 h following type 1 collagen treatment. These results suggest that chicken PDGF-B chain may play an important role in the vascular system and in healing wounded tissue.


http://www.sciencedirect.com/science/article/B6T5X-48XRYVJ-2/2/13b62e08a50b4c73182cdaa5ed29cec9

Despite considerable advances in our understanding of teleost immunity, relatively few cytokine genes, including those for interferon (IFN), have been identified at the molecular level. In contrast, numerous studies have shown that following virus infection or exposure to double-stranded RNA, fish or fish cells produce a soluble factor that is functionally similar to mammalian IFN. A putative catfish (CF) IFN cDNA was identified by BLASTX screening of a catfish EST library generated from a mixed lymphocyte culture enriched for NK-like cells. Consistent with its designation as a putative cytokine cDNA, the 3' non-translated region contained multiple copies of an RNA instability motif. Analysis of the deduced amino acid sequence of CF IFN showed low levels of identity/similarity to a panel of mammalian and avian IFN proteins, and markedly higher similarity to a recently identified zebrafish IFN. To determine if the identified cDNA encoded CF IFN, expression was monitored following infection of channel catfish ovary (CCO) cells with UV-inactivated catfish reovirus or exposure to double-stranded RNA, treatments which induce IFN or IFN-like activity in catfish and other species. In both cases, upregulation of putative CF IFN mRNA was detected. Moreover, upregulation of CF IFN mRNA was accompanied by the appearance of an antiviral factor in the culture medium. To confirm these results, recombinant CF IFN was synthesized in COS-7 cells and shown to have antiviral activity in CCO cells. Collectively, these results argue strongly that the identified catfish cDNA is an IFN homolog.


http://www.sciencedirect.com/science/article/B6T5X-46YXK2B-3/2/1acc4f74e4e13f0453756dc807fd269c

To further characterize genes of immunological importance from non-placental mammals, cDNAs encoding [beta]2-microglobulin ([beta]2m) were isolated from two prototherians, the platypus and an echidna, and one metatherian, a grey short-tailed opossum. In addition, a second allele of [beta]2m was identified in another metatherian species, the brushtail possum. Analysis of the deduced translations revealed conservation of key residues in these molecules over a long evolutionary history. The types of nucleotide substitutions present among the various taxa are also consistent with purifying selection at this conserved locus. An evolutionary tree of [beta]2m was constructed that supports the classic view of evolution with prototherians as the basal mammalian group.

Tumor necrosis factor receptor (TNFR) superfamily regulates diverse biologic functions, including cell proliferation, differentiation, and survival, in addition to providing costimulatory signals for programmed cell death or apoptosis. In this study, cDNA fragments for two distinct TNFR homologues were obtained from a Japanese flounder, Paralichthys olivaceus, cDNA library. Full-length cDNAs of TNFR-1 and TNFR-2 homologues were obtained by using these cDNA fragments as probes. The cDNA for the Japanese flounder TNFR-1 homologue predicts a peptide of 395 amino acids that is 35% identical to the extracellular region of mouse TNFR-1, whereas the cDNA of the Japanese flounder TNFR-2 homologue predicts a peptide of 483 amino acids that is 40% identical to the extracellular region of human TNFR-2. The cytoplasmic domain contains a sequence that has the consensus motif of the death domain of the Japanese flounder TNFR-1 homologue. In a healthy fish, the mRNAs of both TNFR homologues were predominantly expressed in leukocytes, kidney, gill, and spleen. Expression of the Japanese flounder TNFR-1 homologue was induced in peripheral blood lymphocytes (PBLs) after stimulation with LPS (500 [μg/ml] for 1 h, and TNFR-2 homologue was strongly induced in PBLs after stimulation with Con A (50 [μg/ml]) and PMA (0.35 [μg/ml]) for 3 h. The different expression patterns of the two distinct TNFR homologues may be critical in determining whether binding with TNF-[alpha] or TNF-[beta] have activating, proliferative, or apoptotic effects on target cells.


http://www.sciencedirect.com/science/article/B6T5X-40SFFW9-1/2/6534342e242b98a875b96675062116de

The MAGE (Melanoma Associated Antigen) family tumor-specific antigens are shared by a number of histologically different tumors. Till date, only human and mouse MAGE genes have been characterized. Our study describes the first non-mammalian member of MAGE superfamily, DMAGE from D. melanogaster. A conceptual translation of the cDNA of DMAGE identifies a putative protein that contains a motif that shares eight out of nine amino acids with the previously identified promiscuous, HLA-A2 restricted antigenic epitope in the C-terminus of human MAGE-B1 and -B2. Similarly, this motif of DMAGE shares seven out of nine amino acids with the same antigenic epitope of human MAGE-A3 and -A12. Thus, the phylogeny of proteins that activate tumor specific T-cells in mammals as unmutated self-proteins began at least 100 million years earlier in evolution than the emergence of the adaptive immune system of higher vertebrates. Northern analysis revealed that DMAGE is a developmentally regulated gene highly expressed in adult fruit fly and in the embryo of D. melanogaster. In contrast, the expression level of the mRNA of DMAGE in fruit fly larva is substantially lower than in embryo and adult fly. We propose that studies of DMAGE on D. melanogaster may help define the function(s) of MAGE super-family genes.


http://www.sciencedirect.com/science/article/B6T5X-3RYCJTD-3/2/8aaa24a42ca3daa7c897fee86282ffbb5

To further elucidate the cellular mechanisms that mediate programmed cell death in avian
immune cells, differential display analysis was employed to identify differentially expressed genes in chicken thymocytes undergoing apoptosis. Primary cultures of thymocytes were treated with dexamethasone to activate apoptosis and RNA was isolated for differential display analysis. A differential display product designated A1 (479 bp) was identified. This display product was subcloned and induced expression of the genes was confirmed by ribonuclease protection analysis. Nucleotide sequence analysis of A1 revealed a putative 82 amino acid open reading frame that demonstrated limited homology with Bad, an apoptotic regulatory protein. Thus, A1 may represent the avian homolog of Bad.


http://www.sciencedirect.com/science/article/B6T5X-4C9YYD0-7/2/cd1a0cfc00b347726f35bfa16c4a923

The major histocompatibility complex (MHC) class II B locus of the striped bass (Morone saxatilis) was found to contain multiple forms of the class II B gene. Seven complete MHC class II B cDNA clones were isolated and sequenced, identifying five unique allelic forms of a MHC class II B gene. Among three specimens, each representing a geographically distinct population (Chesapeake Bay, MD; Roanoke River, NC; and Santee-Cooper Reservoir, SC) extensive variability was detected in the [beta]1 encoding domain, which corresponds with the functional peptide-binding region (PBR) of known MHC class II molecules. The location of variable amino acid residues in the [beta]1 domains corresponds with polymorphic sites observed in other teleosts and higher vertebrates. The amino acid translated [beta]2 domain encoding regions, transmembrane regions, and cytoplasmic regions of the five clones correlated well with those of known vertebrate MHC class II proteins. Seventy-one percent of the variability found within the presumed PBR encoded at the MHCMosa class II B locus corresponded with that of the PBR of a human MHC class II B gene. Overall, the Mosa sequences showed greatest similarity to the MHC class II B genes of cichlid fishes, as expected from phylogenetic relationships.


http://www.sciencedirect.com/science/article/B6T5X-48JSX1T-1/2/21b740de4452ae3a57acea686da2e31d

A tumor necrosis factor (TNF) [alpha]-like gene, encoding a propeptide of 230 amino acids and a mature (soluble) peptide of 162 amino acids, was identified in channel catfish (Ictalurus punctatus). While the catfish protein shared features in common with both mammalian TNF[alpha] and TNF[beta] homologs, overall sequence identity/similarity was slightly higher vs. TNF[alpha] genes when mature TNF sequences were compared. Phylogenetic analysis placed catfish and other fish TNF sequences within their own cluster apart from mammalian TNF[alpha] and [beta] genes, and supported the suggestion that TNF[alpha] and [beta] genes separated after the divergence of mammals and teleosts. In contrast to trout and carp, but similar to flounder, catfish TNF was present as a single copy gene. Expression studies demonstrated that catfish TNF[alpha] mRNA was present in all tested tissues (i.e. liver, spleen, head kidney, mesonephros, gill, thymus, and PBLs) from an unstimulated fish. Moreover, catfish TNF was constitutively expressed in actively proliferating, but otherwise unstimulated, macrophage (42TA) and T cell (G14D; TS32.17) lines, but not in B cell (1G8 or 3B11) or fibroblast lines. TNF expression was upregulated in PBLs, and in G14D and 42TA cells, but not in 3B11 cells, by PMA/calcium ionophore treatment. These results demonstrate that a catfish homolog of TNF[alpha] has been
identified, and indicate that catfish TNF[alpha] is expressed in catfish in a manner similar to that seen in mammals.

Developmental Biology (15)


During vertebrate embryogenesis, the somites form by segmentation of the trunk mesoderm, lateral to the neural tube, in an anterior to posterior direction. Analysis of differential gene expression during somitogenesis has been problematic due to the limited amount of tissue available from early mouse embryos. To circumvent these problems, we developed a modified differential display PCR technique that is highly sensitive and yields products that can be used directly as in situ hybridisation probes. Using this technique, we isolated NLRR-1 as a gene expressed in the myotome of developing somites but not in the presomitic mesoderm. Detailed expression analysis showed that this gene was expressed in the skeletal muscle precursors of the myotome, branchial arches and limbs as well as in the developing nervous system. Somitic expression occurs in the earliest myoblasts that originate from the dorsal lip in a pattern reminiscent of the muscle determination gene Myf5, but not at the ventral lip, indicating that NLRR-1 is expressed in a subset of myotome cells. The NLRR genes comprise a three-gene family encoding glycosylated transmembrane proteins with external leucine-rich repeats, a fibronectin domain, an immunoglobulin domain and short intracellular tails capable of mediating protein-protein interaction. Analysis of NLRR-3 expression revealed regulated expression in the neural system in developing ganglia and motor neurons. NLRR-2 expression appears to be predominately confined to the adult. The regulated embryonic expression and cellular location of these proteins suggest important roles during mouse development in the control of cell adhesion, movement or signalling.


http://www.sciencedirect.com/science/article/B6WDG-4DJBP6T-1/2/47adc2c79e907615cfb8b87c90af4132

This study presents functional and molecular evidence for acquisition of multidrug transporter-mediated efflux activity as a consequence of fertilization in the sea urchin. Sea urchin eggs and embryos express low levels of efflux transporter genes with homology to the multidrug resistance associated protein (mrp) and permeability glycoprotein (p-gp) families of ABC transporters. The corresponding efflux activity is low in unfertilized eggs but is dramatically upregulated within 25 min of fertilization; the expression of this activity does not involve de novo gene expression and is insensitive to inhibitors of transcription and translation indicating activation of pre-existing transporter protein. Our study, using specific inhibitors of efflux transporters, indicates that the major activity is from one or more mrp-like transporters. The expression of activity at fertilization requires microfilaments, suggesting that the transporters are in vesicles and moved to the surface
after fertilization. Pharmacological inhibition of mrp-mediated efflux activity with MK571 sensitizes embryos to the toxic compound vinblastine, confirming that one role for the efflux transport activity is embryo protection from xenobiotics. In addition, inhibition of mrp activity with MK571 alone retards mitosis indicating that mrp-like activity may also be required for early cell divisions.


http://www.sciencedirect.com/science/article/B6WDG-4816430-5/2/06a20caf404113b84b200ac348198baa

We isolated a full-length cDNA clone of amphioxus AmphiNk2-tin, an NK2 gene similar in sequence to vertebrate NK2 cardiac genes, suggesting a potentially similar function to Drosophila tinman and to vertebrate NK2 cardiac genes during heart development. During the neurula stage of amphioxus, AmphiNk2-tin is expressed first within the foregut endoderm, then transiently in muscle precursor cells in the somites, and finally in some mesoderm cells of the visceral peritoneum arranged in an approximately midventral row running beneath the midgut and hindgut. The peritoneal cells that express AmphiNk2-tin are evidently precursors of the myocardium of the heart, which subsequently becomes morphologically detectable ventral to the gut. The amphioxus heart is a rostrocaudally extended tube consisting entirely of myocardial cells (at both the larval and adult stages); there are no chambers, valves, endocardium, epicardium, or other differentiated features of vertebrate hearts. Phylogenetic analysis of the AmphiNk2-tin sequence documents its close relationship to vertebrate NK2 class cardiac genes, and ancillary evidence suggests a relationship with the Drosophila NK2 gene tinman. Apparently, an amphioxus-like heart, and the developmental program directing its development, was the foundation upon which the vertebrate heart evolved by progressive modular innovations at the genetic and morphological levels of organization.


http://www.sciencedirect.com/science/article/B6WDG-4DFBSN9-2/2/1db3535cfa35454f46f44738590183ff

The Drosophila CNS midline cells constitute a specialized set of interneurons, motoneurons, and glia. The utility of the CNS midline cells as a neurogenomic system to study CNS development derives from the ability to easily identify CNS midline-expressed genes. For this study, we used a variety of sources to identify 281 putative midline-expressed genes, including enhancer trap lines, microarray data, published accounts, and the Berkeley Drosophila Genome Project (BDGP) gene expression data. For each gene, we analyzed expression at all stages of embryonic CNS development and categorized expression patterns with regard to specific midline cell types. Of the 281 candidates, we identified 224 midline-expressed genes, which include transcription factors, signaling proteins, and transposable elements. We find that 58 genes are expressed in mesectodermal precursor cells, 138 in midline primordium cells, and 143 in mature midline cells—50 in midline glia, 106 in midline neurons. Additionally, we identified 27 genes expressed in glial and mesodermal cells associated with the midline cells. This work provides the basis for future research that will generate a complete cellular and molecular map of CNS midline development, thus allowing for detailed genetic and molecular studies of neuronal and glial development and function.

http://www.sciencedirect.com/science/article/B6WDG-4CB083R-4/2/e1989093de162a78044c1f68a1b4b030

In Drosophila, the RNA helicase VASA (VAS) is required for both germ line formation and oocyte differentiation. While the murine VAS homologue is required for spermatogenesis, it is dispensable for germ line formation. The molecular basis for this apparently dual role of VAS in germ line ontogeny is, however, unclear. Recent evidence indicates that fish, like flies, employs VAS both in early and late stages of the germ line development and that there is a sex-linked differential expression of splice variants. We show here that the longer of two splice variants of zebrafish vas is transiently downregulated in the germ line around the time when the germ cells reach the developing gonad. Using transgenic vas:EGFP fish lines, which allow us to distinguish between male and female individuals, we show that the long splice variant reappears in both sexes at around day 25 and is subsequently downregulated during male gonadal development. Our data further suggest that there is a switch from maternal to zygotic expression of the long splice variant of vas as sexual dimorphic development commences.


http://www.sciencedirect.com/science/article/B6WDG-4CX031V-1/2/cc5eb924021be09d9e081576fa513588

Telomerase is critical for the protection of germ line and stem cell chromosomes from fatal shortening during replication. In most organisms, telomerase activity is suppressed in progressively committed cells and falls to basal rates in terminally differentiated lineages. The colonial ascidian Botryllus schlosseri propagates asexually and sexually, presumably from pools of stem cells that self-renew throughout the 2- to 5-year colony life span. Asexual budding takes place continuously from the parental body wall. When the colony reaches a critical size, sexual reproduction commences with the generation of gonads. Here, we establish the existence of 6-15 kb telomeres on the ends of Botryllus chromosomes. We develop a real-time quantitative PCR telomeric repeat amplification protocol (TRAP) assay that reliably detects 0.2-100 TPG units in cells and tissues. We find highest levels of enzymatic activity in the gonads, developing embryos, and tissues containing the earliest asexual buds. Telomerase activity appears to be suppressed in later buds during organogenesis and falls to basal rates in mature zooids. We postulate that this pattern reflects maximum telomere restoration in somatic stem cells of early buds and suppression of telomerase activity in progenitors and terminally differentiated cells, indicative of an alternate role for stem cells as repeated body regenerators in colonial life histories.


http://www.sciencedirect.com/science/article/B6WDG-4C76C1G-1/2/457ba64104db88cb6e178a3c56ed6276

The establishment of neural circuits in the spinal cord depends on the differentiation of functionally distinct types of neurons in the embryonic neural tube. A number of genes have
recently been shown to control the generation of dorsal interneurons through inductive signals provided by the roof plate. The roof plate is a transient signaling center on the dorsal midline of the neural tube that coordinates dorsal CNS development through the action of local peptide signals, primarily the bone morphogenic proteins (BMPs) and the Wingless-related genes (Wnts). The role of the roof plate has become evident through studies of mutations of genes in these gene families, and through several spontaneously occurring mouse mutants, including dreherJ (drJ), all of which cause dorsal neural tube defects. We previously demonstrated that the roof plate is missing in the dreher mouse. Positional cloning of the dreher locus demonstrated that an inactivating point mutation in the LIM homeodomain (HD) transcription factor encoded by the Lmx1a gene, is responsible for the dreherJ phenotype [Nature, 403 (2000) 764]. Here we report that Lmx1a is first expressed at E8.5 in a small number of cells in the lateral neural plate. As the neural tube closes, Lmx1a expression is restricted to the roof plate. In drJ/drJ, although non-functional Lmx1a is correctly expressed at E8.5-E9.5, its expression is lost in the spinal cord roof plate by E10.5. Coincident with the loss of Lmx1a expression, Bmp expression fails, and the generation and differentiation of the dorsal-most spinal cord neurons, the dl1 interneurons, is abnormal. In drJ/drJ embryos, defects are evident in the number of dl1 progenitors, as well as in their migration to form the lateral and medial nuclei, and axon patterning, through mechanisms that apparently involve defects in early steps of neuronal polarity. Consistent with the general hypothesis that a failure of roof plate formation and function results in deficits in dorsal patterning of the neural tube, the dreher affects the generation and differentiation of the dl1 interneuron population.


http://www.sciencedirect.com/science/article/B6WDG-4CP68N2-3/2/202818a0aabb712f8e60d714ca77ba5f

The pha-2 mutant was isolated in 1993 by Leon Avery in a screen for worms with visible defects in pharyngeal feeding behavior. In pha-2 mutant worms, the pharyngeal isthmus is abnormally thick and short and, in contrast to wild-type worms, harbors several cell nuclei. We show here that pha-2 encodes a homeodomain protein and is homologous to the vertebrate homeobox gene, Hex (also known as Prh). Consistent with a function in pharyngeal development, the pha-2 gene is expressed in the pharyngeal primordium of Caenorhabditis elegans embryos, particularly in pm5 cells that form the bulk of the isthmus. We show that in the pha-2 mutant there is a failure of the pm5 cells to elongate anteriorly while keeping their nuclei within the nascent posterior bulb to form the isthmus during the 3-fold embryonic stage. We also present evidence that pha-2 regulates itself positively in pm5 cells, that it is a downstream target of the forkhead gene pha-4, and that it may also act in the isthmus as an inhibitor of the ceh-22 gene, an Nkx2.5 homolog. Finally, we have begun characterizing the regulation of the pha-2 gene and find that intronic sequences are essential for the complete pha-2 expression profile. The present report is the first to examine the expression and function of an invertebrate Hex homolog, that is, the C. elegans pha-2 gene.


Mitogen-activated protein kinase (MAPK) pathways mediate some important cellular processes
and are likely to also regulate preimplantation development. The role of p38 MAP kinase signaling during murine preimplantation development was investigated in the present study. p38 MAPK, p38-regulated or -activated kinase (PRAK; MK5), map kinase-activated protein kinase 2 (MK2), and heat shock protein 25 (hsp25) mRNAs and proteins were detected throughout preimplantation development. Two-cell stage embryos cultured in the presence of SB220025 and SB203580 (specific inhibitors of p38 MAPK [alpha]/[beta]), progressed to the eight-cell stage with the same frequency as controls; however, treated embryos halted their development at the 8- to 16-cell stage. In addition, embryos treated with p38 MAPK inhibitors displayed a complete loss of MK2 and hsp25 phosphorylation and also a complete loss of filamentous actin as indicated by the absence of rhodamine-phalloidin staining. In these inhibitor-treated groups, the embryos were composed of a mixture of compacting and noncompacting cells, and the embryos were one to two cell divisions behind controls. Treated embryos remained viable as the developmental blockade was rescued by removing embryos from the drug treatment and placing them in drug-free medium until they progressed to the blastocyst stage. This study demonstrates that p38 MAPK activity is required to support development through the murine preimplantation interval.


Double-strand RNA (dsRNA)-mediated posttranscriptional gene silencing, also known as RNA interference (RNAi), is a powerful tool to inhibit gene expression in several experimental model systems, including Arabidopsis, Caenorhabditis, and Drosophila. We previously described that the microinjection of Mos dsRNA into fully grown mouse oocytes results in the specific degradation of Mos mRNA in a time- and concentration-dependent manner. We report here a transgenic RNAi approach that is suitable to study gene function during mouse oocyte development and differentiation. The oocyte-specific Zp3 promoter was used to drive the expression of a long hairpin dsRNA (~500 bp) targeting Mos mRNA. Transgenic founder animals appeared healthy, but while males were fertile, females were not, in accordance with the known Mos null phenotype. The amount of Mos mRNA in the transgenic F1 females was reduced by >90%, whereas there was no decrease in the nontargeted tissue plasminogen activator (Plat) mRNA. Moreover, the maturation-associated increase in mitogen-activated protein (MAP) kinase activity was not observed, and the metaphase II eggs underwent spontaneous parthenogenetic activation, thus recapitulating the Mos null phenotype. This approach provides a powerful method to study the functions of any oocyte-synthesized gene during oocyte development and early embryogenesis.


http://www.sciencedirect.com/science/article/B6WDG-4DKTPB0-6/2/9dda2a5a1e9d832ac72202a187dd23f7

Calpactin I, one of the EDTA-extractable proteins of the lens membrane, binds phospholipid and actin in a calcium-dependent manner. It is also a known substrate of the pp60src kinase. Analysis of embryonic chicken lens RNA with a bovine calpactin I-specific cDNA probe revealed the presence of a ~1.8 Kb calpactin mRNA in the lens cells. Six-day embryonic chicken lenses were microdissected into central epithelium, equatorial epithelium, and fiber cells. Total cytoplasmic RNA was isolated from these samples and calpactin I mRNA levels were determined by the polymerase chain reaction (PCR) following reverse transcription (RT). Quantitative PCR indicates
that the calpactin I mRNA levels in the equatorial epithelium are greater than in the central epithelium by a factor of 12.7 +/- 2.7. Calpactin I mRNA in fiber cells is an additional 3.5 +/- 1.5 times greater than in the equatorial epithelium. Whole mounts of embryonic chicken lens epithelia and histological sections of whole lenses were also examined with an antibody directed against chicken calpactin I. Calpactin I was predominantly localized in a punctate distribution in equatorial epithelial cells and near the plasma membrane of elongate fiber cells. The elevated levels of calpactin I mRNA observed in the equatorial epithelium and fiber cells and the immunological localization of the protein suggest a possible role of calpactin I in the elongation of fiber cells during lens differentiation.


http://www.sciencedirect.com/science/article/B6WDG-4CJXT1T-1/2/c599acf524c87adeca7acc1f10f709b3

In ascidian eggs, the existence of several localized maternal cytoplasmic determinants has been proposed and the importance of localized mRNAs for tissue differentiation has been demonstrated. We previously identified the ascidian Y-box proteins (CiYB1, 2 and 3), homologues of which are known to be involved in the storage of maternal mRNA in oocytes of other organisms. In this study, we found that CiYB1 protein is abundant in the gonad, egg, and embryo. Purification of messenger ribonucleoprotein (mRNP) particles from the gonad revealed that CiYB1 was one of their major components. A significant change in the distribution of CiYB1 protein from stored mRNP particles in the gonad to the ribosome fraction in eggs and embryos was observed. This change correlates most likely with the shift of stored maternal mRNAs to polyribosomes. Moreover, we found that CiYB1 colocalized with Cipem and Ci-macho1 mRNAs, which are localized at the posterior end of the embryo at the cleavage stage. Cipem and Ci-macho1 mRNAs were co-immunoprecipitated with CiYB1 in the oocyte and embryo lysates. The formation of a complex between Cipem mRNA and CiYB1 protein resulted in translational repression in the in vitro translation system. Our results indicate that associating with CiYB1 protein contributes to the translational control of the localized mRNA in eggs and embryos.


http://www.sciencedirect.com/science/article/B6WDG-4F030CR-7T/2/fd76d6bdc31e6be20bc5a3ae29764cf0

A major unsolved problem in developmental biology is to determine when and how time- and position-restricted instructions are signaled and received during secondary embryonic inductions such as branching morphogenesis. The mouse embryonic lung rudiment was used to test the hypothesis that endogenous peptide growth factors, specifically epidermal growth factor (EGF), serve as instructive epigenetic signals for morphogenesis. The presence of EGF precursor mRNA transcripts was detected using the reverse-transcriptase-coupled polymerase chain reaction both in E11-E17-day mouse embryo lung tissues in vivo and in E11-day lung cultured for up to 7 days in vitro under chemically defined, serum-free conditions. Immunolocalization identified a position-restricted distribution of EGF in and around the primitive airways both during in vivo lung morphogenesis and in culture. EGF receptors (EGFR) coimmunolocalized with EGF in the primitive airways. Addition of exogenous EGF to lungs in culture resulted in significant concentration-dependent stimulation of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA. Conversely, the addition of tyrphostin
(specific EGF receptor kinase antagonist) to lungs in culture resulted in concentration-dependent inhibition of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA without apparent cytotoxicity. The inhibition of the EGF signal by tyrphostin was confirmed by immunoprecipitation of tyrosine phosphoproteins. We conclude that early mouse embryo lungs express EGF transcripts and corresponding EGF peptides in a specific position-restricted distribution which coimmunolocalizes with EGFR in the primitive airways, while stimulatory and inhibitory studies indicate a functional role for the transduced EGF signal in the epigenetic regulation of lung branching morphogenesis. We speculate that the peptide growth factor EGF serves a function in secondary embryonic morphogenetic inductions, which may be modulated by interaction with other growth factors.


http://www.sciencedirect.com/science/article/B6WDG-47X6RT9-4/2/b6849f3d3c1d9e483dd3318c46280c56

Selected for its high relative abundance, a protein spot of MW ~75 kDa, pl 5.5 was cored from a Coomassie-stained two-dimensional gel of proteins from 2850 zona-free metaphase II mouse eggs and analyzed by tandem mass spectrometry (TMS), and novel microsequences were identified that indicated a previously uncharacterized egg protein. A 2.4-kb cDNA was then amplified from a mouse ovarian adapter-ligated cDNA library by RACE-PCR, and a unique 2043-bp open reading frame was defined encoding a 681-amino-acid protein. Comparison of the deduced amino acid sequence with the nonredundant database demonstrated that the protein was ~40% identical to the calcium-dependent peptidylarginine deiminase (PAD) enzyme family. Northern blotting, RT-PCR, and in situ hybridization analyses indicated that the protein was abundantly expressed in the ovary, weakly expressed in the testis, and absent from other tissues. Based on the homology with PDAs and its oocyte-abundant expression pattern, the protein was designated ePAD, for egg and embryo-abundant peptidylarginine deiminase-like protein. Anti-recombinant ePAD monospecific antibodies localized the molecule to the cytoplasm of oocytes in primordial, primary, secondary, and Graafian follicles in ovarian sections, while no other ovarian cell type was stained. ePAD was also expressed in the immature oocyte, mature egg, and through the blastocyst stage of embryonic development, where expression levels began to decrease. Immunoelectron microscopy localized ePAD to egg cytoplasmic sheets, a unique keratin-containing intermediate filament structure found only in mammalian eggs and in early embryos, and known to undergo reorganization at critical stages of development. Previous reports that PAD-mediated deamination of epithelial cell keratin results in cytoskeletal remodeling suggest a possible role for ePAD in cytoskeletal reorganization in the egg and early embryo.


http://www.sciencedirect.com/science/article/B6WDG-4C4WXCW-2/2/54f3d1692748ceebfd2a41ecd45b5136

The subventricular zone (SVZ) of the developing mammalian forebrain gives rise to astrocytes and oligodendrocytes in the neocortex and white matter, and neurons in the olfactory bulb in perinatal life. We have examined the developmental fates and spatial distributions of the descendants of single SVZ cells by infecting them in vivo at postnatal day 0-1 (P0-1) with a retroviral "library". In most cases, individual SVZ cells gave rise to either oligodendrocytes or...
astrocytes, but some generated both types of glia. Members of glial clones can disperse widely through the gray and white matter. Progenitors continued to divide after stopping migration, generating clusters of related cells. However, the progeny of a single SVZ cell does not differentiate synchronously; individual clones contained both mature and less mature glia after short or long intervals. For example, progenitors that settled in the white matter generated three types of clonal oligodendrocyte clusters: those composed of only myelinating oligodendrocytes, of both myelinating oligodendrocytes and non-myelinating oligodendrocytes, or of only non-myelinating cells of the oligodendrocyte lineage. Thus, some progenitors do not fully differentiate, but remain immature and may continue to cycle well into adult life.

Developmental Brain Research (8)


http://www.sciencedirect.com/science/article/B6SYW-3T46VPV-6/2/b559a1455e84b92eb2338ac68a5b384e

The present study documents the steady-state levels for the mRNAs encoding acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD2) and brain long-chain acyl-CoA synthase (BLACS) during mouse development. It is shown that ACC and FAS mRNA levels are at a maximum 5 days after birth, a time when cell proliferation is intense in the mouse brain, and then decrease steadily to reach 20% of those maximal values at day 20. The ACC transcript isoforms, which were detected in the central nervous system (CNS), originated from promoter P2 of the ACC gene. They encode ACC enzymes which cannot be phosphorylated at the Ser-1200 locus, thus indicating that brain ACC is highly sensitive to citrate activation. The developmental pattern for the SCD2 mRNA level is different from that of true myelin genes, such as CGT. Indeed, the steady-state levels for SCD2 and CGT in 5-day-old brain represent 85% and 5% of their maximal values, respectively. BLACS expression rose during the developmental period studied, but a slow decrease in the mRNA levels was not observed after postnatal day 20, unlike in 'myelin-specific' genes. Therefore, it appears that the expression of the genes involved in fatty acid biosynthesis is independent of the myelinating signal in the mouse CNS.


http://www.sciencedirect.com/science/article/B6SYW-3R37WNP-1/2/cf43206befbb6fd975407cd1903a65

Previous studies have demonstrated the critical role glutamate plays in the hypothalamus, both in the developing and adult brain. The expression of metabotropic glutamate receptor (mGluR) mRNA (mGluR1-8) was studied in the suprachiasmatic (SCN) and arcuate (ARC) nuclei. Using reverse Northern blots and cDNA-PCR, we found that all eight cloned mGluRs were expressed in these brain regions. Most had not previously been detected here. Surprisingly, this included mGluRs that had previously been thought to be restricted to the retina, such as mGluR6. We also
detected, cloned, and sequenced a splice variant of mGluR7 (mGluR7b). Developmentally, the age of maximal expression of mGluRs was dependent on the region. For instance, mGluR5 was more strongly expressed in neonatal ARC than in adult, whereas the opposite was true in the SCN. Compared with P10 neonates, mGluR1, R3, R6, R7a, R7b, and R8 showed a greater expression in adult SCN and ARC.


http://www.sciencedirect.com/science/article/B6SYW-3TT60NV-5/2/a39ad79f62b55c7afb1e2d4636723697

Using degenerate primers designed to amplify genes containing homeodomains, we have used reverse transcription and polymerase chain reaction to amplify and clone a rat homeobox gene. Based on the nucleotide and predicted amino acid sequences, the rat cDNA clone contains a high degree of sequence similarity to murine genes which are members of the paired-like class of homeobox genes (Ptx2, Otlx2, solurshin and Ptx1). Considering the high degree of sequence similarity and similar restricted expression patterns, we have named the cloned rat gene rPtx2 (rat Ptx2 homolog). Northern analysis revealed two rPtx2 transcripts expressed in the developing rat brain. Yet, only a single gene was detected by Southern blot hybridization, suggesting that multiple messages are the result of alternative transcriptional initiation, splicing or processing of a common message. The expression pattern of rPtx2 was further delineated by in situ hybridization to rat embryos. Within the brain, tissue specific expression was observed in the differentiating neural cells of the posterior hypothalamus, tegmentum, and rhombomere r1. Expression was also observed in the developing pituitary, maxilla, mandible, tongue and umbilical cord. To further study the control of Ptx2 gene expression, we used an in vitro model for neural differentiation by treating mouse embryonic stem cells with retinoic acid. Within 24 h and prior to detection of a neural phenotype in the culture, murine Ptx transcripts were induced and remained elevated for at least 6 days. This suggests that retinoic acid may be an important inductive signal which regulates the developmental and tissue-specific expression of Ptx2.


http://www.sciencedirect.com/science/article/B6SYW-485P97H-42/2/3a35235cede58fa0902dce1d485089879

We have examined the central nervous system (CNS) of developing and adult transgenic mice carrying sequences upstream of the histone H1[deg] gene fused to the E. coli [beta]-galactosidase gene (lac Z). The transgene is induced in a subset of the neuronal population during postnatal development, coinciding with neuronal terminal differentiation. At postnatal day 9, the earliest time at which the transgene product can be detected, positive neurons are observed in the granular layer of the cerebellar cortex and in the pyramidal fields of the hippocampus. The transgene is then induced in other areas of the CNS, such as the neocortex, thalamus, hypothalamus, olfactory bulb, globus pallidus superior and inferior colliculus, substantia nigra, pontine nuclei and brain stem. Induction is unrelated with determination and quiescence, which are essentially prenatal. The overlapping of the temporal and regional patterns of transgene activity with those of the endogenous protein shows that the accumulation of H1[deg] in differentiating neurons is at least in part under transcriptional control. In the light of these results, the H1[deg] gene appears as the only mammalian histone gene that specifically responds to terminal differentiation. However, not all terminal differentiated neurons express H1[deg] at detectable levels. For instance, Purkinje cells are negative. In neurons, terminal differentiation
Gap junction coupling between neurons is important for the temporal and spatial co-ordination of neocortical development and can be visualised by dye-coupling. Neuronal dye-coupling in the rat neocortex is extensive during the first 2 postnatal weeks and diminishes rapidly thereafter. We used RT (reverse transcriptase)-PCR to investigate the time-related changes in mRNA expression for the connexins (Cx) Cx 26, Cx 30, Cx 32, Cx 36, Cx 37, Cx 40, Cx 43, Cx 45 and Cx 46 as well as for [beta]-actin and GAPDH in rat neocortex during the first 6 postnatal weeks. The time courses for mRNA expression for GAPDH, Cx 30, Cx 36 and Cx 43 were also investigated by northern blotting. Cx 30 and Cx 45 mRNA abundance showed no time-dependent changes during the early postnatal period. The relative abundance of Cx 32, Cx 43 and Cx 46 mRNA increased significantly during the first 2-3 weeks and then remained relatively constant during weeks 3-6. The relative abundance of Cx 26, Cx 36, Cx 37 and Cx 40 mRNA also increased significantly during the first 10-15 postnatal days but then declined significantly from their peak values during weeks 3-6. [beta]-actin mRNA expression showed no time-related changes but GAPDH mRNA expression increased significantly during the first postnatal week, then remained constant. The time-dependent changes in mRNA relative abundance for GAPDH, Cx 36 and Cx 43 determined by northern blotting corroborate the results from the RT-PCR study. None of the Cx exhibited time-dependent changes in mRNA expression in homogenates of rat neocortex which parallel the changes in neuronal dye-coupling during postnatal development.


The expression of the amyloid precursor protein (APP) gene has been examined in the basal forebrain of rats from birth to adulthood. Levels of total APP mRNA are highest at birth and at postnatal day 15 (P15). The most abundant transcript in rat brain in APP-695, whose expression has previously been found to be largely restricted to the central nervous system. Comparison of the developmental profiles of APP-695 mRNA with that of Kunitz-protease inhibitor (KPI)-containing APP mRNA shows that the greatest difference in expression occurs at P15, when APP-695 message levels are over 6-fold higher than KPI-containing APP mRNA (APP-751, APP-770). This is the largest difference in the APP-695/KPI-APP ratio observed during postnatal development and coincides with the period of maximal neurotrophic responsiveness in the basal forebrain. These results suggest that the APP gene is alternatively spliced during postnatal development and that regulated expression of APP-695 may be influenced by neurotrophic factors in vivo.

To test the hypothesis whether a failure to express neurotrophins or a neurotrophin receptor might underlie the pathology observed in mutant mice with degeneration of regionally distinct subpopulations of neurons, the expression of BDNF, NT-3, TrkB, TrkC and synaptophysin mRNA was examined in the cerebellum of mutant lurcher (lc/+ ) and weaver (wv/+)/(wv/wv) mice. To identify the expression patterns of individual neurons, we used in situ hybridization with digoxigenin labeled ribonucleotide probes. RT-PCR of cerebellar mRNA for BDNF, NT-3, TrkB and TrkC (GAPDH as internal standard) was performed in parallel. Although especially in homozygous (wv/wv) weaver mice the normal anatomical order and number of the cerebellar neurons is grossly disturbed, residual Purkinje and granule neurons of both mutants displayed a normal expression pattern of the neurotrophins examined. Thus, the affected animals showed no significant signal decrease compared to healthy littermates or C3H mice. Our results suggest that the loss of specific neuron populations in the cerebellum of either mutant occurs via mechanisms either independent or downstream of the neurotrophins examined in this study.


Fetal alcohol exposure is the most common nonhereditary cause of mental retardation in the western world. Rats prenatally treated with ethanol liquid diet exhibit extensive defects in the brain that accurately model those observed in humans. To analyze the ethanol effects on gene expression during brain development, we performed mRNA differential display and two-dimensional electrophoresis on gestational day (G) 13 and G16 brain from rats treated with ethanol liquid diet. Using mRNA differential display followed by a variety of quantitative analyses, three genes were confirmed to be ethanol-responsive. Among them was Neuroendocrine-Specific Protein-A (NSP-A), which is known to be affected by thyroid hormone in the cortex at this developmental time. However, two additional genes known to be thyroid hormone-responsive were unaffected by ethanol, indicating that interference with thyroid hormone action may not be a predominant pathway by which alcohol induces damage in the fetal brain. The observation that interferon-inducible protein-10 (IP-10) is up-regulated in ethanol-treated fetal brain may indicate the presence of a disease process recruiting CD8+ T-cells capable of interfering with myelination. The result of two-dimensional (2D) electrophoresis and Western analyses demonstrated that few changes in the abundance of individual proteins or the phosphorylation of proteins at threonine and tyrosine were induced by prenatal ethanol exposure. A critical analysis of the approaches used in the present study may be important for future studies in this field.

Summary
Inductive interactions between gut endoderm and the underlying mesenchyme pattern the developing digestive tract into regions with specific morphology and functions. The molecular mechanisms behind these interactions are largely unknown. Expression of the conserved homeobox gene Barx1 is restricted to the stomach mesenchyme during gut organogenesis. Using recombinant tissue cultures, we show that Barx1 loss in the mesenchyme prevents stomach epithelial differentiation of overlying endoderm and induces intestine-specific genes instead. Additionally, Barx1 null mouse embryos show visceral homeosis, with intestinal gene expression within a highly disorganized gastric epithelium. Barx1 directs mesenchymal cell expression of two secreted Wnt antagonists, sFRP1 and sFRP2, and these factors are sufficient replacements for Barx1 function. Canonical Wnt signaling is prominent in the prospective gastric endoderm prior to epithelial differentiation, and its inhibition by Barx1-dependent signaling permits development of stomach-specific epithelium. These results define a transcriptional and signaling pathway of inductive cell interactions in vertebrate organogenesis.

Diabetes Care (2)


OBJECTIVE--Pioglitazone is a member of the thiazolidinediones (TZDs), insulin-sensitizing agents used to treat type 2 diabetes. The aim of this study was to define the effect of pioglitazone on the expression of genes related to carbohydrate and lipid metabolism in subcutaneous fat obtained from type 2 diabetic patients. RESEARCH DESIGN AND METHODS--Forty-eight volunteers with type 2 diabetes were divided into two groups treated for 12 weeks with placebo or pioglitazone (30 mg/day). The expression of several genes was quantified by real-time RT-PCR. RESULTS--Pioglitazone treatment increased the expression of genes involved in glycerol-3-phosphate synthesis. The mRNA expression of PEPCK-C and glycerol-3-phosphate dehydrogenase (GPDH) increased (P < 0.01) in patients treated with pioglitazone. There was no difference in glycerol kinase (GyK) mRNA levels. The expression of genes that regulate fatty acid availability in adipocytes, including lipoprotein lipase (LPL) and acetyl-CoA synthetase (ACS), was higher (P < 0.01) in pioglitazone-treated patients. Pioglitazone stimulated (P < 0.0001) expression of c-Cbl-associated protein (CAP), whereas tumor necrosis factor-(alpha), leptin, resistin, angiopoietin like-4, and 11-[beta]-hydroxysteroid dehydrogenase type 1 (11{beta} HSD 1) were not affected by pioglitazone. The baseline peroxisome proliferator-activated receptor (PPAR)-(gamma)1 mRNA was significantly correlated with mRNA for LPL, CAP, ACS, 11{beta} HSD 1, GyK, fatty acid synthase, leptin, and GPDH, whereas PPAR-(gamma)2 mRNA was correlated with CAP, PEPCK-C, leptin, and GPDH. CONCLUSIONS--Treatment with pioglitazone increased body weight, and this is associated with upregulation of some, but not all, genes previously demonstrated as "TZD responsive" in subcutaneous fat. The results suggest that TZDs might increase body weight through the upregulation of genes facilitating adipocyte lipid storage in vivo.
OBJECTIVE--To clarify the role of the T-lymphocyte-associated-4 (CTLA-4) polymorphism in the susceptibility to child-onset type 1 diabetes with regard to its clinical characteristics and complications with autoimmune thyroid disease (AITD) in the Japanese population. RESEARCH DESIGN AND METHODS--The CTLA-4 49 A/G polymorphism was detected by the PCR-restriction fragment-length polymorphism (RFLP) method in 97 type 1 diabetic subjects and 20 patients with Graves' disease, a cohort which included 4 patients who also had type 1 diabetes. RESULTS--The genotypes and allele frequencies of this polymorphism did not differ between the type 1 diabetic subjects and the control subjects. The G allele frequency was 63.9% in the type 1 diabetic subjects. The G allele frequency in the subgroup of patients with a high titer of autoantibodies to the GAD antibody (Ab) was 72.9% (P = 0.0499 vs. control subjects); in the subgroup of patients without HLA DRB1*0405, it was 72.6% (P = 0.0271 vs. control subjects); and in the subgroup of patients with a residual beta-cell function, it was 78.6% (P = 0.0391 vs. control subjects). The G allele frequency in the patients with Graves' disease was also significantly higher at 78.1% (P = 0.0405 vs. control subjects). Furthermore, the frequency in our diabetic subjects complicated with Graves' disease was even higher (87.5%). CONCLUSIONS--We have demonstrated that a distinct association exists between the G allele of CTLA-4 and high values of GAD Ab, residual beta-cell function, and the absence of HLA-DRB1*0405.

Diabetes Research and Clinical Practice 54(2):95.


The aim of this study was to investigate whether an association exists between the angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism and microvascular complications of type 2 diabetes mellitus in Turkish patients. A total of 239 type 2 diabetic patients and 138 sex and age matched control subjects were included into the study. The I/D polymorphism was determined by polymerase chain reaction (PCR). Nephropathy status was determined according to urinary albumin/creatinine ratio (μg/mg) (300 macroalbuminuria) and retinopathy was evaluated by fundoscopic examination and by fluorescein fundus angiography. The distribution of ACE I/D polymorphism and allele frequencies in diabetic patients were not significantly different from controls, DD genotype 32.2 versus 37.2%; ID genotype 50.6 versus 47.1%; and II 17.2 versus 15.2%; D allele 57.5 versus 61.2%; I allele 42.5 versus 38.8%. Genotype distribution between normo-, micro- and macroalbuminuric patients did not differ significantly (DD:ID:II (%), normoalbuminuria, 35:46:19; microalbuminuria, 28:55:17; macroalbuminuria, 31:55:14). There was also no difference in genotype distribution between patients with and without retinopathy (DD:ID:II (%), retinopathy positive, 32:51:17; retinopathy negative, 33:49:18). In conclusion, the ACE I/D polymorphism does not seem to be associated with diabetic nephropathy and retinopathy in Turkish type 2 diabetic patients.

Oxidative stress and the gene expression at the transcriptional level of antioxidant enzymes were investigated in two models of diabetes in mice. We used KKAY mice as a model of obese insulin-resistant diabetes, and streptozotocin-induced diabetic mice (STZ mice) as a model of insulin-deficient diabetes. C57BL mice and saline-injected ICR mice were used as the respective non-diabetic controls. To assess oxidative damage, plasma malondialdehyde (MDA), urine 8-isoprostanate and 8-hydroxy deoxyguanosine (8-OHdG) were measured. The mRNA expression of antioxidant enzymes, superoxide dismutase 1 (SOD-1) and glutathione peroxidase 1 (GPx-1) in the kidney and heart were quantified using a real-time polymerase chain reaction. The KKAY mice demonstrated moderate hyperglycemia and hyperlipidemia, and the STZ mice showed severe hyperglycemia and hypolipidemia. The KKAY mice, but not the STZ mice, showed elevated plasma MDA relative to the non-diabetic controls. Urine 8-isoprostanate and 8-OHdG in both diabetic mouse groups increased significantly. The urine oxidative stress markers in the severely hyperglycemic STZ mice were higher than those in the moderately hyperglycemic KKAY mice. Although GPx-1 and SOD-1 showed elevated mRNA expression in the KKAY mice in the kidney and heart, in the STZ mice they did not increase compared to the controls. The compensatory up-regulation of the mRNA expression of antioxidant enzymes may be impaired in the insulin-deficient severely hyperglycemic state.


We investigated the relationship between advanced diabetic retinopathy (ADR) and an angiotensin-converting enzyme (ACE) gene polymorphism in subjects with type 2 diabetes and ADR, pre-proliferative (PrePDR) or proliferative diabetic retinopathy (PDR) without overt nephropathy. Polymerase chain reactions were used to detect insertion/deletion (I/D) polymorphisms of the ACE gene. There was no difference in the frequency of II, ID, or DD genotypes, or of I and D alleles among subjects with type 2 diabetes without diabetic retinopathy (NDR) or with simple diabetic retinopathy (SDR) and non-diabetic controls. There was also no difference in the frequency of ACE genotypes among subjects with type 2 diabetes with NDR, or SDR and ADR. However, the frequency of the ACE DD genotype in ADR was significantly higher than that in controls ([chi]2=6.64, P=0.036). On the other hand, the frequency of the D allele in ADR was significantly higher than that in controls ([chi]2=6.33, P=0.012), NDR ([chi]2=4.18, P=0.041) and SDR ([chi]2=4.89, P=0.027), respectively. These results indicate a significant relationship between the presence of the D allele polymorphism in the ACE gene and ADR in Japanese subjects with type 2 diabetes and no overt nephropathy.

Recently Iwata et al. reported that the polymorphism in NeuroD exon 2 (Ala45Thr) was associated with adult-onset Type 1 diabetes in Japanese. Furthermore, the mutations in the NeuroD as a regulator of insulin transcription have been reported to result in Type 2 diabetes. We, therefore, aimed to clarify the role of this Ala45Thr polymorphism in the susceptibility to Type 1a, immune-mediated, diabetes of child-onset Japanese patients. Eighty patients with child-onset Type 1 diabetes were examined along with 121 non-diabetic subjects as the controls. The polymorphism in Ala45Thr was defined using the PCR-RFLP method. The GAD Ab, IA-2 Ab, HLA-DRB1 genotypes and residual [beta]-cell function at 3 years from onset were evaluated in relation to the difference in this polymorphism. The frequency of the Ala45Thr heterozygotes was significantly higher in the Type 1 diabetic patients than in the controls (21.3 versus 9.9%, P=0.0252). The frequency of loss of [beta]-cell function was higher in heterozygotes patients than in wild type homozygotes patients (P=0.0112). Type 1 diabetic patients with DRB1*0901 allele showed a significantly higher frequency, 27.9%, of the Ala45Thr variant than the controls (P=0.0041). In conclusion, the Ala45Thr polymorphism contributes to the risk of development of, and to the early deterioration of [beta]-cell function, in Type 1a diabetes among the Japanese population.


http://www.sciencedirect.com/science/article/B6T5Y-42BSNV3-3/2/1348bc5b63c6cb0cbc24e7294fa8ba51

A case-control study to investigate whether the aldose reductase (AC)n dinucleotide polymorphism (termed 5'-ALR2 polymorphism) is useful as a genetic marker for risk of microvascular complications among Caucasians Type 1 diabetic patients in Australia is reported. This marker was amplified from patient genomic DNA and then fractionated in 5% formamide-urea gels. A total of nine alleles was observed with Z, Z-2 and Z+2 being the major alleles. The distribution of alleles was comparable in diabetic subjects with diabetes and microvascular complications, diabetes without complications and normal non-diabetic control subjects. Similarly, when the distribution of alleles was examined in the patients subcategorized according to the presence of diabetic nephropathy or diabetic neuropathy, no significant association was observed. While the size of the study makes it impossible to exclude a weak linkage, it is concluded that the 5'-ALR2 polymorphism is not useful as a genetic marker for susceptibility to diabetic microvascular complications in Caucasian Type 1 diabetic patients.


http://www.sciencedirect.com/science/article/B6T5Y-3XK6TFR-8/2/34a4d28d96276e8534610e0125a7a8b5

To clarify risk factors for the progression of microalbuminuria in Japanese type 2 diabetic patients, the longitudinal study for 10 years was conducted on 67 outpatients with type 2 diabetes, who had shown no overt proteinuria at baseline. The urinary albumin index (UAI) has been determined based on the mean of at least two random urine samples each year. Categories were defined as normoalbuminuria (UAI =300.0). Progression was defined as worsening of the category and/or more than doubling of the baseline UAI value. Multiple logistic regression
analysis was performed using age, duration of diabetes, HbA1c, blood pressure, BMI, serum lipids, smoking habits, and alcohol consumption as independent variables and the progression of microalbuminuria as a dependent variable. Age and HbA1c were estimated as significant and independent variables. Furthermore, genetic polymorphisms of angiotensin I-converting enzyme (ACE) and angiotensinogen were analyzed to evaluate the genetic contribution. The D/D genotype of ACE was significantly more common in progressors than in non-progressors. These results suggest that glycemic control and age are important risk factors and the D/D genotype of ACE acts as a risk factor for the progression of microalbuminuria in Japanese type 2 diabetic patients.


Variants of calpain-10 gene (CAPN 10) have recently been reported to be associated with type 2 diabetes (T2DM). Haplotype combination 112/121 defined by three single nucleotide polymorphisms (SNPs) (UCSNP-43, -19 and -63) of CAPN 10 conferred the highest risk for T2DM in Mexican-Americans. In this study, we aim to examine whether these genetic variants contribute to the susceptibility for T2DM in a Chinese population. The frequencies of these three SNPs were determined in 168 patients with T2DM and 104 controls. Distribution of alleles, genotypes and haplotypes at three loci were not significantly different between the two groups. No difference was observed in the 112/121 haplotype combination distribution. However, haplotype combination 112/221 was more prevalent in the control group than in T2DM group (16.35% versus 7.14%, p = 0.025). Control subjects with haplotype combination 112/121 had higher serum cholesterol level than others without haplotype combination 112/121 (5.7 [plus-or-minus sign] versus 5.2 [plus-or-minus sign] 0.7, p = 0.011). Our results suggest that haplotype combination 112/221 associated with reduced risk for T2DM and haplotype combination 112/121 might be a risk factor for increased serum cholesterol in Chinese population.


In order to clarify the nature of T lymphocytes infiltrating the pancreatic islets of patients with insulin-dependent diabetes mellitus (IDDM), we analysed T cell receptor (TCR) gene transcripts expressed in pancreatic biopsy specimens of patients with recent-onset IDDM. We also investigated the expression of cytokines (interferon-[gamma]; IFN-[gamma]; tumour necrosis factor-[alpha]; TNF-[alpha]; interleukin-4: IL-4; interleukin-6: IL-6) in the same specimens. The TCR V[beta] repertoire was not restricted either in the pancreas or the peripheral lymphocytes of IDDM patients. In contrast, the TCR V[alpha] repertoire was restricted in the pancreas, but not in the peripheral blood lymphocytes, of IDDM patients. The sequence analysis of the complementarity-determining region 3 (CDR3) of the TCR[alpha] revealed the presence of dominant clonality in [alpha] chains of T cells in the patients. IFN-[gamma] mRNA was highly expressed in the pancreas of IDDM patients, while IL-4 mRNA was deficient. A lower level of expression of IL-6 mRNA was detected in the IDDM pancreas than in the control tissue. These results indicate that T cells bearing a distinct TCR[alpha] chain are selectively retained and activated within the pancreas of recent-onset IDDM.

http://www.sciencedirect.com/science/article/B6T60-3RXYC61-3/2/c78a7c1620545384eb06544f0541675f

Quantitative-competitive polymerase chain reaction (QPCR) was performed on serial sputum samples from 22 consecutive cases of acid fast bacilli (AFB) smear-positive pulmonary tuberculosis. Of 94 specimens, 55, 72, and 83% were positive by culture, AFB smear, and QPCR, respectively. Of 52 culture-positive specimens, 6% were negative by PCR, and 13% were negative by AFB smear. Of 42 culture-negative specimens, AFB smear and QPCR were positive in 55 and 61%, respectively. AFB smear and QPCR results were strongly correlated (r = 0.75, p < 0.001), but each correlated less strongly with culture (r = 0.54, p < 0.005 for smear and R = 0.52, p < 0.005 for QPCR). When patients were classified by microbiologic response, responders tended to have less DNA in their sputum and shorter time to a negative PCR result compared to nonresponders. These data do not suggest a great advantage of QPCR over AFB smear for predicting culture results in patients with pulmonary tuberculosis.


http://www.sciencedirect.com/science/article/B6T60-421TKTG-6/2/5bbf4cfbc2bf174cbb651c966684b77b

The nature and frequency of mutations in the rpoB gene of rifampin-resistant clinical Mycobacterium tuberculosis isolates vary considerably according to geographical locations. There is no information on the prevalence of specific mutations in clinical M. tuberculosis strains isolated from patients in Middle-Eastern countries. In this study, 13 rifampin-resistant and 6 susceptible clinical M. tuberculosis isolates were tested for identification and characterization of mutations in the rpoB gene by INNO-LiPA Rif. TB kit and DNA sequencing of the PCR amplified target DNA. The kit identified all six susceptible strains as rifampin-sensitive and the DNA sequence of the amplified rpoB gene in the target region matched perfectly with the wild-type sequence. The kit identified 12 resistant isolates as rifampin-resistant with specific detection of mutations in 8 isolates while one of the rifampin-resistant strain was identified as rifampin-susceptible. DNA sequencing confirmed these results and, in addition, led to the specific detection of mutations in 4 rifampin-resistant isolates in which specific base changes within the target region could not be determined by the INNO-LiPA Rif. TB kit. The majority (8 of 13) of resistant isolates involved base changes at codon 531 of the rpoB gene. Mutations at codon position 531 within the rpoB gene have also been reported in majority of rifampin-resistant strains from Greece and St. Petersburg, Russia but not from other geographical locations.

http://www.sciencedirect.com/science/article/B6T60-47F4R44-6/2/602005e45f1049c15c51c27211d63888

Mutations conferring resistance to rifampin in rifampin-resistant clinical Mycobacterium tuberculosis isolates occur mostly in the 81 bp rifampin-resistance-determining region (RRDR) of the rpoB gene. In this study, 29 rifampin-resistant and 12 -susceptible clinical M. tuberculosis isolates were tested for characterization of mutations in the rpoB gene by line probe (INNO-LiPA Rif. TB) assay and the results were confirmed and extended by DNA sequencing of the PCR amplified target DNA. The line probe assay identified all 12 susceptible strains as rifampin-sensitive and the DNA sequence of RRDR in the amplified rpoB gene from two isolates matched perfectly with the wild-type sequence. The line probe assay identified 28 resistant isolates as rifampin-resistant with specific detection of mutation in 22 isolates including one isolate that exhibited retro-resistance containing both the wild-type pattern as well as a specific mutation within RRDR while one of the rifampin-resistant strain was identified as rifampin-susceptible. DNA sequencing confirmed these results and, in addition, led to the specific detection of mutations in 5 rifampin-resistant isolates in which specific base changes within RRDR could not be determined by the line probe assay. These analyses identified 8 different mutations within RRDR of the rpoB gene including one novel mutation (S522W) that has not been reported so far. The genotyping performed on the isolates carrying similar mutations showed that majority of these isolates were unique as they exhibited varying DNA banding patterns. Correlating the ethnic origin of the infected TB patients with the occurrence of specific mutations at three main codon positions (516, 526 and 531) in the rpoB gene showed that most patients (11 of 15) from South Asian region contained mutations at codon 526 while majority of isolates from patients (6 of 11) of Middle Eastern origin contained mutations at codon 531.


http://www.sciencedirect.com/science/article/B6T60-4DS878B-6/2/3496eb8ddcaa20814e314e27bad00ffc

We have developed a real-time PCR assay for detection of Trypanosoma brucei DNA in human blood samples. The PCR was conducted with newly designed primers targeting the 177-bp repeat satellite DNA in T. brucei and with Sybr Green to monitor the amplicon accumulation. DNA purification using Chelex 100(R) resin was performed on blood samples collected on Whatman FTA(R) cards and was shown to be a simple and quantitative method as revealed by real-time PCR. The detection limit of the assay was 100 trypanosomes per mL blood, corresponding to an analytical sensitivity of 0.1 genome equivalents. Trypanosome DNA was detected in all blood samples from sleeping sickness patients and, furthermore, the identity of the amplicon was confirmed in all assays by dissociation analysis. Although template DNA from blood samples was amplified with significantly lower efficiency than genomic DNA, similar efficiency between all assays ensured quantitative results. No amplicon product was obtained with samples from uninfected individuals. The results indicate that the real-time PCR assay described is a rapid and sensitive method suitable for the detection of T. brucei in human blood samples in routine clinical laboratory practice.

Bialek, R., N. Binder, et al. (2002). "Comparison of fluorescence, antigen and PCR assays to detect Cryptosporidium parvum in fecal specimens." Diagnostic Microbiology and Infectious Disease
To optimize routine screening for cryptosporidiosis, 198 stool samples from patients at risk and from calves were examined by enzyme immunoassay (EIA), a direct fluorescent-antibody (DFA) and a modified immunofluorescence assay. Ninety-nine samples were positive in at least one assay, whereas 99 were negative in all three assays. Sensitivity of antigen EIA and DFA were similar (94%, 95% CI: 88-98%, and 91%, 95% CI: 84-95%). The modified immunofluorescence was significantly less sensitive (64%, 95% CI: 55-74%). 149 samples were also examined by two nested PCR assays targeting either the 18S rRNA or Cryptosporidium outer wall protein (COWP) gene. A PCR product was amplified from 86 out of 89 samples being positive in at least one other assay (sensitivity 97%, 95% CI: 91-99%). None was obtained from 60 samples negative in the three other assays. PCR assays did not increase the detection rate. Antigen EIA or DFA appear sufficient for routine Cryptosporidium screening of fecal samples.


Strains of Chlamydophila pneumoniae may be associated with respiratory disease or atherosclerosis. Two real-time quantitative PCR assays targeting the species-specific genes Cpn0278 and ArgR were developed to compare the in vitro growth of respiratory strains AR39 and K6 with that of atherosclerotic strain A03 and to quantify C. pneumoniae in clinical samples. A third real-time PCR assay was designed to assess contamination with Mycoplasma spp. The assays targeting C. pneumoniae detected DNA concentrations corresponding to 10^4 to 10^4 inclusion-forming units (IFU)/reaction and were highly specific. AR39 exhibited the longest lag phase and period of exponential growth; K6 augmented growth rates at higher inocula; and A03 grew at highest rates. Contamination with Mycoplasma spp. of AR39 and A03 unlikely accounted for growth differences between them. Numbers of IFU in C. pneumoniae-positive respiratory secretions varied within 4 to 5 orders of magnitude. The assays described may prove valuable for pathogenicity studies.


Polymerase chain reaction and cytotoxin assays were performed to identify as Helicobacter pylori type I (cagA+/tox+) or type II (cagA-/tox-) 56 (59.6%) strains from 94 patients. Of these patients 64 were affected by nonulcer dyspepsia (NUD), 10 by gastric ulcer (GU), 19 by duodenal ulcer (DU), and 1 by both GU and DU. H. pylori strains were tested for cagA using two sets of primers; target sequences were detected in 40-42/56 (71.4-75%) depending on the set of primers used, while cytotoxin-producing strains (tox+) were 26/56 (46.4%). Tox+ strains were isolated in 13/32 (40.6%), 2/7 (28.6%), and 11/17 (64.7%) in NUD, GU, and DU patients, respectively. However, the different percentage between cagA+ strains from NUD patients (13/32; 40.6%) and patients
with ulcerative diseases (13/23; 54.2%) is not statistically significant ($p = 0.462$). Because the two sets of primers employed for amplification of cagA target sequences give different results, we concluded that cagA alone could not be taken as predictive factor for severity of gastroduodenal disease. It has been found that H. pylori type I is associated with duodenal ulcer disease.


http://www.sciencedirect.com/science/article/B6T60-44SJYVYH-8/2/c8d1fe22cf2a649fade3484aad164d

Several reports have evidenced geographic differences in the prevalence of vacA (vacuolating cytotoxin gene) alleles and cagA (cytotoxin-associated gene) status among Helicobacter pylori isolates. We investigated the occurrence of these virulence-associated genes status among our isolates, and their relationship with ulcer disease outcome. Besides, ureA-B polymorphism was studied. One hundred isolates, comprising 32 from patients with ulcer disease (UD) and 68 from patients with non-ulcer dyspepsia (NUD), were analyzed. Eighty-four percent of isolates were cagA-positive without statistically significant difference in prevalence between patients with UD or NUD. Genotype vacA-s1m1 was predominant, although unlike other South American regions, subtype s1am1 occurrence was higher than s1b. The multivariate model used to estimate the predictive value of cagA and vacA status for UD development disclosed infection with vacA-s1am1 isolates as the only variable that increased the risk of UD onset. ureAB fingerprinting showed considerable genetic divergence among isolates, however, confirmed that certain DNA banding profiles are conserved worldwide.


http://www.sciencedirect.com/science/article/B6T60-3RH7HTT-2/2/74c20cf661a87cfcf10e44ac8893f718d

Acinetobacter spp. isolates were increasingly obtained from clinical specimens and sterility samples, and a subsequent epidemiological investigation implicated an intermittently contaminated supply of commercially acquired enrichment broths. Typing was performed with DNA amplification by the polymerase chain reaction (PCR) using enterobacterial repetitive intergenic consensus sequence primers, ERIC2 and reverse ERIC1R. The reliability of this PCR-based typing method was verified by the ability of the technique to demonstrate homology and differences among isolates from an epidemiologically well-defined pseudo-outbreak.


http://www.sciencedirect.com/science/article/B6T60-476TVR0-4B/2/8ce832d2c8702688985d44a8825cccc

The structural gene encoding the 10-kD antigen from Mycobacterium tuberculosis was amplified
by the polymerase chain reaction. The 297-base-pair (bp) product was detected among 45 strains representing 14 mycobacterial species, but was absent from 11 species related to the mycobacteria. The gene was localized to a ~2000-bp SstII restriction fragment of the organisms' chromosomes.


A multiplex real-time polymerase chain reaction (RT-PCR) targeting the mecA and nuc genes was developed for the detection of methicillin resistance and identification of Staphylococcus aureus. Novel mecA and nuc primers and fluorescence resonance energy transfer hybridization probes specific for the mecA and nuc genes were evaluated. The assay was performed using the LightCycler system (Roche Molecular Biochemicals, Mannheim, Germany) and evaluated against the traditional gel-based multiplex PCR (PCR-gel) method currently used at Royal Perth Hospital. Clinical isolates (n = 222) and isolates from a culture collection library (n = 206) were tested by both assays in parallel. The RT-PCR assay was 100% sensitive and specific for the detection of methicillin resistance and for the identification of S. aureus when compared with the PCR-gel assay. Results from the RT-PCR assay showed 5 isolates with lower efficiency fluorescence curves for the nuc gene PCR fragment. DNA sequencing showed mutations within the region of the probe-binding sites compared with the reference strain. The results of the RT-PCR assay were available within 2 h. This rapid mecA/nuc RT-PCR assay is a suitable and practical tool for the routine detection of methicillin resistance and identification of S. aureus, which can be easily incorporated into the diagnostic molecular microbiology laboratory work flow.


This study was conducted to assess the reliability of a commercial enzyme-linked viral inducible system (ELVIS) (Diagnostic Hybrids, Inc., Athens, OH) for rapid detection and typing of herpes simplex virus (HSV). Results using ELVIS were compared to those of shell vial culture (SVC) and HSV detection with monoclonal antibodies and an immunoperoxidase stain plus typing with MicroTrak direct fluorescent antibodies (Trinity Biotech PLC, Wicklow, Ireland). Specimens yielding discrepant HSV results were tested by polymerase chain reaction (PCR); those with discrepant typing results were stained with Simulfluor (Chemicon, Temecula, CA). Of the 206 samples tested, 144 were negative and 54 were HSV-positive by both methods (agreement, 96.1%). Five specimens were positive by ELVIS but negative by SVC; 3 of these were positive and 2 were negative by HSV PCR. Both of the latter were the result of mechanical problems early in the study. Three specimens were positive by SVC but negative by ELVIS; all 3 were positive by HSV PCR. After resolution of discrepancies, the sensitivity and specificity for detection of HSV were 95.0% and 100% for SVC, respectively, and 95.0% and 98.6% for ELVIS. Of the 46 HSV-positive samples that were typed, 26 were called type 2 and 18 were type 1 by both methods (agreement, 95.7%). The 2 specimens with discrepant results were called HSV-2 by SVC, staining with MicroTrak, and HSV-1 with ELVIS; both of these were type 2 when stained with the Simulfluor reagent. ELVIS is a reliable alternative to SVC for rapid detection and typing of HSV.

http://www.sciencedirect.com/science/article/B6T60-49SM23P-2/2/580007b85e1b2186e5e0836533b85edd

A small but significant proportion of blood cultures processed by the BACTEC 9000 series systems is signaled positive, while subsequent Gram's stain and culture on solid media yield no pathogens. In this study, 15 "false-positive" vials (7 aerobes, 8 anaerobes) from 15 patients were investigated for the presence of bacteria and fungi by eubacterial 16S rDNA and panfungal 18S rDNA amplification, respectively. All samples turned out negative by both methods. Most patients (7) had neutropenia, which does not support the theory that high leukocyte counts enhance the generation of false-positive results. In conclusion, the results of this study indicate that false-negative results generated by the BACTEC 9000 series are inherent to the automated detection and not due to the growth of fastidious organisms.


http://www.sciencedirect.com/science/article/B6T60-3XP0GJB-5/2/1236bcb8fe17429a1df082efc2253d76

Six Candida dubliniensis isolates were recovered from two HIV-infected individuals in the course of a prospective study of recurrent oral candidosis among HIV-positive patients in Spain. Candida albicans strains as well as non-albicans strains were also obtained from these two patients. C. dubliniensis strains were germ-tube-positive and produced abundant chlamydospores. Fingerprinting the genomic DNAs of these six C. dubliniensis with the C. albicans-specific probe 27A as well as karyotyping was performed to confirm the identification of these isolates. Further analysis of their genomic DNAs was performed by PCR-fingerprinting with the core sequence of phage M13, and they exhibited species-specific multilocus band patterns, clearly distinct from those of C. albicans isolates analyzed in this study and in a previous one (Diaz-Guerra 1997). Intraspecies variation was also seen among PCR patterns yielded by C. dubliniensis isolates from different patients. Although few strains have been analyzed, the use of this PCR-fingerprinting procedure is a promising tool for further epidemiologic studies with C. dubliniensis. The isolation of C. dubliniensis from Spanish HIV-infected patients contributes to the idea of widespread geographic distribution of this species.


http://www.sciencedirect.com/science/article/B6T60-49BXMF8-1/2/c9bb633ece0e36548e36b366d267c047

The Roche MagNA Pure automated nucleic acid extraction system was tested for its ability to extract Borrelia burgdorferi DNA from a diverse set of spiked specimen types including blood, cerebral spinal fluid, synovial fluid, urine and ticks. A method comparison between MagNA Pure
automated extraction and manual extraction, using either QIAamp columns or phenol/chloroform extraction, showed equivalent detection sensitivities for all methodologies with all specimen types (except for urine, in which case QIAamp extraction was twofold less sensitive). Eighty positive clinical specimens (as determined by an independent testing method), including 76 synovial fluid, and 4 cerebral spinal fluid specimens, were found to be positive by the MagNA Pure/real-time PCR method of extraction and detection. This data shows that the MagNA Pure system can be used to extract B. burgdorferi DNA from clinical specimens, and when combined with real-time PCR, the result is an extremely sensitive assay with limited hands on time and rapid turn around times.


http://www.sciencedirect.com/science/article/B6T60-405KCXV-1/2/15f1cef77b053da9828d09aa7fe114

To improve tools for the surveillance of invasive H. influenzae in the context of the drastic decrease of type b infections following the implementation of vaccination, a two-step PCR technique was developed to detect the capsule and type specific regions of H. influenzae. The technique of Falla et al (1994) was modified to amplify in a first step the capsule and type b regions by multiplex PCR. For non-b capsulated strains, the type a, c, d, e, and f loci were afterward detected simultaneously by an optimized touch-down PCR technique. An internal control of extraction and amplification (16S rDNA) was included for both PCR techniques. Overall, this technique was shown to perform as efficiently or better than the slide agglutination without risks of interpretation errors. Of the 138 H. influenzae strains tested, seven that had given doubtful results by the agglutination technique were unequivocally typed by PCR.


http://www.sciencedirect.com/science/article/B6T60-3VH0WSC-5/2/dc876aa69dd5a2d251b8fed3b4ed486

A set of 47 Austrian human, food, and veterinary Escherichia coli O157:H7 isolates was used to evaluate five different epidemiological typing methods. Ribotyping using an automated microbial characterization system (RiboPrinter(TM)) was not suitable for detection of epidemiological relatedness. All but one E. coli strain were typeable by phage typing. Random amplified polymorphic DNA-PCR fingerprinting was performed using primer M13 containing the sequence 5'-GAG GTG GGC GGT TCT-3' and primer 1247 (5'-AAGAGCCCGT-3'). Although both methods recognized only two clusters, both dendrograms grouped most of the EHEC O157 isolates into epidemiologically related subgroups. Pulsed-field gel electrophoresis of XbaI digested total DNA was a valuable subtyping system. We found that major differences can exist between results of multiple subtyping methods. E. coli O157 isolates should not be classified as epidemiologically related or nonrelated on the basis of a single typing method alone.

The minimum inhibitory concentrations (MICs) of 18 antibiotics were determined for 66 clinical isolates of staphylococci. Genotypes, mutations in the quinolone resistance-determining regions (QRDRs), and effect of efflux were determined in the 18 levofloxacin-resistant isolates, for which the MICs of levofloxacin were high (\(\geq 8\) \([\mu g/ml]\)). The increased levofloxacin resistance mainly resulted from some combinations of mutations in the QRDRs, although NorA-mediated efflux may play a minor role in resistance. A combination of mutations in GrlA (Ser80Phe), GrlB (Pro451Ser), and GyrA (Ser84Leu) was found in 4 methicillin-resistant Staphylococcus aureus (MRSA) isolates that were unrelated genotypically. The mutations in grlA QRDR varied in the isolates classified as being in an identical pulsed-field gel electrophoresis (PFGE) group, although the grlB, gyrA, and gyrB QRDRs were the same. These results suggest that the patterns of amino acid mutations in the QRDRs can provide distinct epidemiologic information from PFGE genotypes in fluoroquinolone-resistant MRSA. A combination of at least three mutations in GrlA, GrlB, and/or GyrA is required to increase the MICs of fluoroquinolones, although all of the levofloxacin-resistant MRSA retained the MICs of sitafloxacin in the range of 1 to 2 \([\mu g/ml]\).


http://www.sciencedirect.com/science/article/B6T60-43F3W7B-3/2/239af0bf24c32a49025848527f8d5bf

A hexon-based fluorogenic polymerase chain reaction (PCR) assay utilizing the 5'-nuclease activity of DNA Taq polymerase was developed as a rapid and type-specific diagnostic system for adenovirus type 4 (Ad4) detection and quantification. The assay consists of a pair of flanking primers and an internal fluorescence labeled probe that allows real time amplification to quantify the Ad4 virus. One out of 12 flanking primer pairs evaluated (combinations of three forward primers and four reverse primers) was found to be optimal for Ad4 virus detection that yielded background-free operation, i.e., no fluorescent signal generated by non-template controls. The assay was employed to detect Ad4 reference virus strain RI-67, Wyeth Ad4 vaccine strain and 71 different clinical Ad4 isolates from US military recruits used in this study with consistent sensitivity (lower detection limit) of 2-4 pfu per PCR reaction. The assay showed linear Ad4 detection with a dynamic range of greater than five logs (from 2-4 pfu/assay to greater than 105 pfu/assay). This Ad4-specific assay did not crossreact with representative members of Ad subgroups A, B, C, D and F at viral concentrations greater than 108 pfu/ml. It was also demonstrated that Ad4 viruses could be efficiently detected from throat swabs (71/72 specimens or 98.6% detection sensitivity) of infected patients by the Ad4-specific PCR. In general, there was a good correlation between PCR determined viral titers in throat swabs and time required to detect viral cytopathic effects (CPE) in cell culture. Evaluation of the simple Ad4 specific assay developed in this study could be used to provide a rapid clinically relevant diagnosis of Ad4 infections in patients with acute respiratory disease (ARD).

A novel ceuE-based multiplex PCR system was developed as an efficient diagnostics test to detect and differentiate C. jejuni and C. coli. There is no cross reactivity between C. jejuni and C. coli. In addition, the assay does not produce a positive signal from other enteric bacteria including Salmonella, Shigella and Escherichia coli strains. Campylobacter detection sensitivity was determined to be equivalent to previously reported PCR for other enteric bacteria. We also noticed that silicon dioxide extraction can improve Campylobacter detection sensitivity from infected stool samples. It was demonstrated that the PCR assay developed in this study had a much better Campylobacter detection rate than the traditional culturing method (77% versus 56%). However, we also identified small numbers of culture positive stools (8%, or 16 out of 202 samples) that did not yield PCR positive results for Campylobacter. These PCR negative/culture positive stools were proven to be inhibitory to PCR amplification.


http://www.sciencedirect.com/science/article/B6T60-3XG80VC-5/2/9747beff63fd866e099c38ae7d70f7d

We have evaluated a PCR technique using primers based on Pneumocystis carinii major surface glycoprotein (MSG) genes, a multicopy gene family, for utility in detection of P. carinii in BAL and oropharyngeal samples obtained from immunosuppressed patients. These primers were able to detect P. carinii DNA in as little as 16 fg of genomic DNA. PCR using MSG primers detected P. carinii DNA in 7 smear-positive BAL samples (100% sensitivity), and found no P. carinii DNA in 12 smear-negative BAL samples (100% specificity). Mitochondrial ribosomal RNA (mrRNA) primers, commonly used in PCR studies of PCP, detected P. carinii in six of seven positive samples (85.7% sensitivity) and none of 12 were negative samples (100% specificity). Diagnosis of PCP by amplification of 81 oropharyngeal samples using MSG primers had a 50% sensitivity (4/8) and 96% specificity (70/73). PCR with mrRNA primers was 37.5% sensitive (3/8) and 100% specific (73/73). All three false-positive MSG results showed a very low intensity on Southern hybridization. PCR using MSG gene primers should prove valuable in the diagnosis of PCP.


http://www.sciencedirect.com/science/article/B6T60-476TVR0-4C/2/fbabe5916856b98935f9b8ff64c5e5b0

The polymerase chain reaction (PCR) and automated DNA sequencing were used to detect a genetic locus, rpoB, associated with rifampin resistance in Mycobacterium tuberculosis (TB) in clinical isolates and directly in clinical specimens. Primers derived from the sequence of a TB rpoB gene fragment were used to amplify DNA from bacterial and mycobacterial isolates. An rpoB-specific PCR product was obtained for five of five TB, seven of eight other mycobacterial species. Nocardia sp., Corynebacterium sp., Streptomycys sp., Actinomycys sp., and Rhodococcus sp., but not for 15 isolates (eight genera) representing usual bacterial flora. Sequence comparison of the amplified rpoB region revealed the occurrence of TB-specific "signature nucleotides" at three positions. PCR yielded amplification products for seven of 16 clinical specimens. Five of the seven contained TB-specific DNA, as well as sequences that predicted rifampin susceptibility in accord with agar dilution results. None of ten specimens that were culture negative for TB yielded TB-specific PCR products. These results with a limited number of clinical specimens demonstrate the feasibility of direct detection by PCR of rifampin-
resistant TB in clinical specimens. Such testing may serve as a rapid surrogate test for multidrug-resistant TB in laboratories with PCR and automated sequencing capability.


http://www.sciencedirect.com/science/article/B6T60-3XG80VC-3/2/3040e91bdc80d70bd26a1a178744c7ab

This study evaluated a polymerase chain reaction (PCR) method for detection of methicillin-resistant Staphylococcus aureus (MRSA) in specimens referred for nosocomial surveillance. PCR was used to detect the mecA and nuc gene targets using yellow growth on mannitol salt agar containing 6 mg/liter oxacillin (MSO-6) as a source of DNA (N = 645). The diagnostic values for PCR compared with culture methods were 97% specificity, 100% sensitivity, 96% positive predictive value, and 100% negative predictive value. Total cost for PCR per test is $3.62 compared to $4.77 for culture. However, the total cost per specimen is significantly lower due to only 20% of all surveillance specimens producing yellow colonies on MSO-6. The average turnaround time for the PCR method is 48 h compared with 82 h for culture. PCR amplification of mecA and nuc genes using yellow colonies on MSO-6 is a simple, fast, accurate and cost-effective method for routine use in clinical laboratories for detecting MRSA in surveillance specimens.


A patient case report describes an Enterococcus faecium strain isolated from a blood culture that was resistant to linezolid (MIC, 8 [μg/mL; G2576U mutation of 23S rRNA]. Co-resistances were identified for vancomycin, ampicillin, macrolides, fluoroquinolones, chloramphenicol, rifampin, gentamicin (high-level), nitrofurantoin and trimethoprim/sulfamethoxazole. Etest (AB BIODISK, Solna, Sweden) and disk diffusion results also detected the oxazolidinone resistance pattern. Laboratories should be aware of the rare possibility of these strains occurring during linezolid therapy or spontaneously (this case) in contemporary practice, and have in vitro susceptibility methods available capable of detecting oxazolidinone resistance.


http://www.sciencedirect.com/science/article/B6T60-45YCN21-4/2/0718a382b98619dce85f10a59c05439b

We sampled commensal yeasts from three body sites of 24 healthy individuals to examine the patterns of commensal yeast species distribution and strain relatedness within and among individuals. To examine the short-term dynamics, each individual was sampled three times every 35-40 days at each of three body sites: mouth, fingernail, and toenail. The hosts included six
genealogically unrelated individuals and 18 that belonged to four families. A total of 63 morphologically distinct colonies were isolated, identified, and genotyped. Nine yeast species were recovered, including 28 isolates of Candida albicans; 26 of C. parapsilosis; 2 each of C. krusei and C. tropicalis; and 1 each of C. famata, C. glabrata, C. guilliermondii, C. lusitaniae and Trichosporon beigelii. A significant difference in total yeast recovery rate between families was observed. However, body sites did not differ in the rates of yeast recovery. The three body sites showed different species distributions with the fingernail sample containing the highest species diversity, followed by the toenail sample. The oral sample contained the lowest species diversity with all 23 oral isolates being C. albicans. Among the 63 strains, forty-six unique genotypes were identified by PCR fingerprinting. Eleven shared-genotypes were identified, seven of which were from the same body site of the same host. The other four were from different members of the same family. Several family-specific genotypes and genotype clusters were found but the results were inconsistent with strict familial transmission of human commensal yeasts. A single host can have multiple species or multiple genotypes of the same species at the same or different body sites. Changes of species and genotypes over the sampling period for the same body site of individual hosts were also observed, including one direct observation of familial yeast transmission between two members of the same family during our sampling period. Our results indicate dynamic processes of yeast colonization, maintenance and evolution in healthy human hosts.


http://www.sciencedirect.com/science/article/B6T60-3X231S4-6/2/6a9b64c83146838bd390a2e113f55588

A simple PCR set-up for the detection of cytomegalovirus in clinical specimens was developed. All components of the PCR master mix including Taq DNA polymerase, uracil N-glycosylase, and primers were preformulated and stored frozen in aliquots. After thawing the master mix aliquots, the PCR was immediately started after the addition of sample DNA. This method gave excellent reproducible PCR results without loss of enzyme activities following storage at -20[°]C for at least 4 months.


http://www.sciencedirect.com/science/article/B6T60-48TK61T-1/2/7eb253f70e65d1d6fe4d75e7617f18b3

Phenotypic identification of fungi in clinical microbiology laboratories is often difficult and late, especially for slow growing and rarely encountered fungi. We describe the application of 18S ribosomal RNA (rRNA) gene sequencing in the early diagnosis of a case of Exophiala peritonitis. A yeast-like fungus was isolated from the dialysate fluid of a 66-year-old man undergoing continuous ambulatory peritoneal dialysis. It grew slowly after 12 days of incubation to yield mature cultures to permit recognition of microscopic features resembling those of Exophiala, a dematiaceous mold. 18S rRNA gene sequencing provided results 12 days earlier than phenotypic identification and revealed 15 base difference (0.9%) between the isolate and Exophiala sp. strain GHP 1205 (GenBank Accession no. AJ232954), indicating that the isolate most closely resembles a strain of Exophiala species. The patient responded to 4 weeks of intravenous amphotericin B therapy. Early identification of the fungus was important for the choice of anti-fungal regimen. As opportunistic fungal infections in immunocompromised patients
are globally emerging problems, the development of molecular techniques for fungal identification is crucial for early diagnosis and appropriate treatment.


http://www.sciencedirect.com/science/article/B6T60-4DYTXHH-3/2/ef7429e13fe5d2d84cf13b5a007dece7

The human gastrointestinal tract harbors an extremely diverse and complex microbial ecosystem. Most of the existent data about the enteric microflora have been generated using stool samples, but the collection and storage of fecal samples are often problematic. The influence of the storage of stool samples on the bacterial diversity and the degradation of bacterial DNA was analysed in this study. Stool samples from 5 healthy volunteers were exposed to different storage temperatures and durations. The bacterial diversity and the amount of intact bacterial DNA were analysed by single-stranded conformation polymorphism analysis (SSCP) and real-time polymerase chain reaction (PCR), both using a 16S rDNA approach. Additionally, biopsy specimens were taken from 3 of the 5 individuals to compare fecal and mucosal flora. The bacterial diversity of the fecal flora and the total number of bacteria were significantly reduced after 8 and 24 hours at both room temperature and 4\[deg\]C. The mucosa-associated bacterial microflora showed substantial differences compared with the fecal flora. The observed alterations of fecal flora during storage point to the difficulty of the molecular analysis of the bacterial diversity and the enumeration of bacterial cells in fecal samples.


http://www.sciencedirect.com/science/article/B6T60-436VYWP-6/2/9d76b42af82d7ab73230c111fb701548

Pneumocystis carinii pneumonia (PCP) remains a major cause of morbidity and mortality in immunocompromised patients, including those infected with human immunodeficiency virus (HIV). The advent of real-time PCR technology offers the potential for rapid PCR results for the detection of P. carinii. In this report we describe the modification and evaluation of an existing PCR-based method for the detection of P. carinii DNA, into a real-time PCR assay suitable for use with the LightCycler system. Twenty eight induced sputum and bronchial washing specimens from 28 patients were tested by both a conventional PCR assay and a real-time PCR assay. Twelve specimens (42.9%) were positive in both the conventional and real-time PCR assays and sixteen (57.1%) were negative in both assays. The melting points of the amplified P. carinii DNA product obtained by melting curve analysis by the LightCycler of all P. carinii positive specimens ranged from 81.5[deg]C to 83.9[deg]C. There were no discordant results between the two assays for any of the specimens tested and results were available within 2 h for the real-time PCR assay compared to up to 11 h for the conventional PCR assay.

This study was designed to characterize H. pylori from pediatric gastric biopsy specimens in terms of several genes (vacA, cagA, cagE, iceA1, iceA2, and babA2) proposed to be involved in the pathogenesis of this organism. Many of these genes have been studied in adult H. pylori isolates, however, these genes have not been well characterized in H. pylori from children. Using PCR we observed that 44% of the H. pylori in our biopsies shared two common genotypes (vacA s1b m1, cagA, cagE, iceA2 +/- babA2). While 26% of the H. pylori had unique genotypes. The cag pathogenicity island associated genes, cagA and cagE, were found together in 64% or our H. pylori, while 84% were iceA2 positive. The presence of the babA2 gene has been proposed to be associated with a higher risk of H. pylori related diseases, however, we found that only 36% of our H. pylori contained this gene.


A model of acute disseminated Candida albicans infection in New Zealand rabbits was developed to determine the sensitivity and accuracy of polymerase chain reaction (PCR) assay compared with the lysis-centrifugation blood culture method. Primers used amplify a DNA fragment from the multicopy gene coding for the small subunit rRNA, highly conserved in fungi. The sensitivity of PCR achieved in rabbit blood samples spiked with Candida albicans was 10-50 CFU/100 [mu]L. A nested-PCR increased the limit of detection 10-fold. The sensitivity achieved exclusively with the lysis-centrifugation method (37.5%) was higher than that obtained with PCR (25%), but lower than nested PCR (52.5%). The combination of both techniques, lysis-centrifugation and nested PCR, increased the overall sensitivity rate to 62.5%. These results have demonstrated that, globally, the nested PCR was more sensitive than both single PCR and lysis-centrifugation culture in detecting C. albicans in blood from immunocompetent rabbits with acute disseminated candidosis. PCR could be a useful complementary technique to traditional methods in the early diagnosis of candidemia.


Reliable detection of methicillin resistance in coagulase-negative staphylococci (CNS) is required for appropriate therapy of serious infections from these pathogens. To determine the most accurate method of measuring methicillin resistance in CNS initially reported as methicillin susceptible by automated methods, we compared mecA detection by polymerase chain reaction (PCR) with phenotypic methods. One hundred eighty-eight blood culture isolates of CNS that were initially reported as susceptible to methicillin using commercial methods (Vitek or MicroScan) were tested by agar dilution, disk diffusion, oxacillin salt agar screen plate, and a multiplex PCR assay using primer sets for mecA and 16S rRNA. Sixteen isolates (8.5%) previously reported as methicillin susceptible by automated methods contained the mecA gene. MICs of these isolates ranged from 0.5 [mu]g/mL to >=128 [mu]g/mL. Ten of these isolates had
MICs equal to or below the NCCLS breakpoint of 2 \(\mu\)g/mL. Six of the 10 isolates (4 with MICs of 0.5 \(\mu\)g/mL and 2 with MICs of 2 \(\mu\)g/mL) did not grow on any of the oxacillin screen plates after 48 h of incubation at 30\(^\circ\)C or 35\(^\circ\)C. All six isolates were induced to grow in the presence of oxacillin at 128 \(\mu\)g/mL by serial passaging on plates containing increasing concentrations of antibiotic. Retesting with MicroScan and Vitek detected methicillin resistance in 7 and 10 isolates, respectively. Disk diffusion testing with incubation for 48 h proved to be the next best method after PCR for detection of methicillin resistance (15 of 16 isolates). Commercial automated methods and some methods recommended by National Committee for Clinical Laboratory Standards may not detect methicillin resistance in CNS that carry the mecA gene and have MICs just below breakpoint.


http://www.sciencedirect.com/science/article/B6T60-3RXYC61-1/2/1c3147fba2e228bc0630a144bea17125

The opportunistic pathogen Pneumocystis carinii (PC) is a frequent cause of a life-threatening pneumonia in human immunodeficiency virus (HIV)-infected individuals and in other immunocompromised hosts. Specimens obtained from 128 bronchoalveolar lavage (BAL) fluid samples from 123 HIV-positive patients with pulmonary disease and undergoing a diagnostic bronchoscopy were evaluated to detect this organism. We have developed a rapid DNA extraction procedure for nested polymerase chain reaction (PCR) using two sets of primers (pAZ102-E, pAZ102-H and P1 = 5'-CTAGGATATAGCTGGTTTTC-3' and P2 = 5'-TCGACTATCTAGCTTATCGC-3'). The results were compared using cytological techniques (direct wet mount, Giemsa, toluidine blue O) and related to the clinical follow-up of patients. The nested PCR had a 91% sensitivity and a 93% specificity. The effect of chemoprophylaxis and the evaluation of the follow-up of patients are discussed. Nested PCR may represent an important additional tool, along with current cytological methods, for the detection of P. carinii; however, at present it cannot replace routine microbiological methods more simple and less expensive.


http://www.sciencedirect.com/science/article/B6T60-3YCM0KG-C/2/f38e299856a88da140ac044e0b5d0c0f

Current laboratory diagnosis of Lyme borreliosis relies on tests for the detection of antibodies to Borrelia burgdorferi with known limitations. By using a simple extraction procedure for urine samples, B. burgdorferi DNA was amplified by a nested PCR with primers that target the specific part of the flagellin gene. To control possible inhibition of the enzyme (polymerase), a special assay using the same primers was developed. We examined 403 urine samples from 185 patients with skin manifestations of Lyme borreliosis. Before treatment, B. burgdorferi DNA was detected in 88 of 97 patients with Lyme borreliosis. After treatment, all but seven patients became nonreactive. Six of these seven persons suffered from intermittent migratory arthralgias or myalgias, and one from acrodermatitis chronica atrophicans. Two of 49 control patients with various dermatologic disorders and none out of 22 presumably healthy persons were reactive in the PCR. In addition to urine, breast milk from two lactating women with erythema migrans was tested and also found reactive. Borrelia burgdorferi DNA can be detected with high sensitivity (91%) by a nested PCR in urine of patients with Lyme borreliosis. In addition, this test can be a
reliable marker for the efficacy of treatment.


Control of Bordetella pertussis in the community is hampered by slow and insensitive diagnostic tests. We therefore examined the accuracy and cost of culture, direct fluorescent antibody (DFA) staining, and PCR in a routine clinical laboratory. Six hundred thirty seven nasopharyngeal swabs and aspirates in casamino acids transport medium were cultured, stained with polyclonal (Difco), and monoclonal (BL-5 and Accu-Mab) anti-B. pertussis reagents, and amplified by an IS481-specific PCR. PCR products were detected by a hybridization-enzyme immunoassay kit (Gen-eti-k DEIA, DiaSorin), with confirmation by a second PCR in a separate laboratory. Sensitivities and specificities of culture, polyclonal DFA, monoclonal DFA, and PCR were 36 and 100%, 11.4 and 94.6%, 8.3 and 98.4%, and 95.0 and 99.3%, respectively, with a prevalence of 15.7%. The DFA tests were the most economical, and the PCR cost was 31% higher than culture. This study suggests that with minor improvements in economy, pertussis PCR can be implemented in a clinical laboratory with marked improvement in diagnostic accuracy.

http://www.sciencedirect.com/science/article/B6T60-405KCXV-4/2/a9f255ac8a2e6beaef207bd67e677dcd


http://www.sciencedirect.com/science/article/B6T60-448J95T-5/2/e7ecd8894e3640ef1378e7c1362b0911

The polymerase chain reaction (PCR) offers one of the most sensitive methods for detecting Cryptosporidium parvum but its sensitivity in fecal material can be greatly reduced by a variety of poorly defined 'inhibitors'. Methods that separate the parasitic oocyst from fecal material prior to DNA extraction circumvent inhibitor interference but are problematic for frozen specimens since 'intact' oocysts are required for separation. We report here a relatively low-cost, rapid method for extracting C. parvum DNA from frozen fecal materials that can be used in a PCR assay for detection of single parasitic oocysts.

http://www.sciencedirect.com/science/article/B6T60-4809R8S-1/2/a13dbe477a567834447864d90365e5b4

Atypical pathogens such as Chlamydia pneumoniae, Legionella pneumophila and Mycoplasma pneumoniae are an important cause of community-acquired pneumonia. The available detection methods (culture and serology) either lack sensitivity or give only a retrospective diagnosis. In order to improve their detection and quantification in respiratory samples, a real-time multiplex PCR, performed in two separate reactions, was developed for these three pathogens. The comparison of multiplex real-time and conventional PCR assay on 73 respiratory specimens...
showed an overall agreement of 98.3%, corresponding to 95.8%, 100% and 100% agreement for C. pneumoniae, L. pneumophila and M. pneumoniae, respectively. Clinical application of this multiplex real-time PCR was done on 40 respiratory samples from 38 patients with respiratory tract infections. Of 19 serology-positive patients, 14 were confirmed by the multiplex real-time PCR to be infected by either one of the three pathogens. All samples from serology-negative patients were negative with the multiplex real-time PCR.


A novel polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis (PRA) of the hsp65 gene was used for the routine identification of mycobacteria in a high throughput clinical laboratory. A total of 2036 clinical isolates were tested by PRA in conjunction with other methods. The PRA identification of M. tuberculosis complex was 100% sensitive and specific, and 74.5% of nontuberculous mycobacteria (NTM) were correctly identified. It gave highly consistent results for Mycobacterium avium complex (MAC) species and for most isolates of M. fortuitum, M. chelonae, and M. kansasii. It had proven to be highly robust and stable despite usage on such a large-scale and is thus particularly suitable for use in high throughput laboratories in areas with a high incidence of tuberculosis.


A biotinylated single-tube nested polymerase chain reaction (PCR) assay with microwell hybridization assay (bPCR-ELISA) was developed for detection of Mycobacterium tuberculosis in clinical specimens. A total of 659 specimens (601 respiratory specimens and 58 nonrespiratory specimens) were collected for evaluation using three DNA amplification techniques: newly designed bPCR-ELISA, in-house single-tube nested PCR for IS6110 gene sequence (nPCR), and commercial automated assays, the Cobas Amplicor System from Roche Diagnostic Systems (aPCR). Sixty-four (9.7%) specimens were culture-positive for M. tuberculosis. Eleven (1.7%) specimens culture-positive for nontuberculosis mycobacteria were negative by all three PCR assays. The resolved performance of bPCR-ELISA, nPCR, and aPCR was found at sensitivities of 97%, 94%, and 97%, respectively. All three PCR assays exhibited a 100% specificity. In evaluation of bPCR-ELISA, a clear distinction between PCR-positive and PCR-negative specimens when an OD405 value of 0.6 was chosen as cut-off. With serial dilutions of M. tuberculosis H37Rv DNA, the detection limit of bPCR-ELISA was found to be 0.75 cfu per reaction at OD405 value of 0.6. Our developed bPCR-ELISA provides a highly sensitive and low-costing molecular diagnosis suitable for developing countries with high prevalence of tuberculosis.

A retrospective study including 515 Mycobacterium tuberculosis isolates from 215 patients was conducted to investigate possible laboratory contamination with M. tuberculosis over a 1-year period in a university hospital. All cultures underwent variable-number tandem-repeat (VNTR) typing. Cultures suspected of being contaminated in the VNTR analysis and possible sources of contamination underwent mycobacterial interspersed repetitive unit (MIRU) typing further. Overall, 8 (3.7%) cases of 215 patients were considered possible false-positives. Five (2.3%) cultures might be contaminated during initial batching processing, and 1 (0.5%) and 4 (1.9%) cultures of them were further classified as presumed and possible cases, respectively, of cross-contamination on clinical grounds. Three (1.4%) cultures might be contaminated by cultures that had been processed in species identification procedures in the same laminar-flow hood. The 2-step strategy using VNTR and MIRU analyses in combination in this study appears to be a valuable means for the study of false-positive cultures.


Alterations of the APC, K-ras, and beta-catenin genes are defined as early events in colorectal tumorigenesis. These alterations are well-known as constituents of Vogelstein's pathway, however, the relationship among them is unclear. For understanding colorectal tumorigenesis it is important to evaluate their relationship. We analyzed the relationship between beta-catenin and K-ras gene mutations in clinical colorectal samples. Sixty-four cases of colorectal cancers (44 proximal, 20 distal) without a family history of colorectal cancer were used for this study. We purified genomic DNAs from fresh surgical samples and, thus, analyzed the mutations of beta-catenin (exon 3) and K-ras (codons 12 and 13) by PCR direct sequencing method using Big Dye terminator cycle sequencing with AmpliTaq polymerase FS. We found 27% (17/64) K-ras mutations (proximal 25%, 11/44; distal 30%, 6/20). The frequency of beta-catenin mutations was 11% (7/64; proximal 9%, 4/44; distal 15%, 3/20). All cases with beta-catenin mutation had no mutation of K-ras. All sites of beta-catenin mutation have been reported previously (codons 33, 34, 41, 45). In cell lines, it has been reported previously that beta-catenin and K-ras play the same roles in activation of cyclin D1 transcription. Our results may support this report and suggest that some colorectal cancers with beta-catenin mutation will progress without K-ras mutation. Further study may disclose a new pathway or new mechanism of colorectal tumorigenesis.

http://www.sciencedirect.com/science/article/B7582-4B41037-7/2/ed0650d64b7ce2b850d4fdce45756a45

Background. Kupffer cells, monocytes and infiltrating T cells have been considered the major source of interleukin-1[beta] and tumour necrosis factor-alpha in the liver. Aims. To explore the expression of interleukin-1[beta] and tumour necrosis factor-alpha and to evaluate the density and the distribution of T lymphocytes and monocytes/macrophages in the liver of patients with primary and secondary tumours. Methods. Tumoural and peritumoural liver samples were examined from 21 patients with hepatocellular carcinoma, 1 D with hepatic metastases, 5 with benign focal liver lesions and 4 healthy adult livers. Interleukin-1[beta] and tumour necrosis factor-alpha mRNAs were detected by a semiquantitative comparative reverse transcriptase polymerase chain reaction. T lymphocytes and monocytes/macrophages were detected by immunohistochemistry. Results. Higher levels of interleukin-1[beta], tumour necrosis factor-alpha, CD3+ and CD68+ cells were found in the tissue surrounding hepatocellular carcinoma and metastases than in the tumour itself. A strong expression of CD68+ and CD3+ cells was found mainly along the tumour-host interface but the highest expression of CD3+ cells was found at the metastasis interfaces. Interleukin-1[beta] expression, CD3+ and CD68+ cell densities were higher in peritumoural samples than in so-called "normal" liver tissue. Conclusions. An increased production of interleukin-1[beta] and, to a lesser extent, of tumour necrosis factor-alpha mRNA coincides with the presence of cancer, be it primary or secondary, both in healthy and cirrhotic livers. The presence of cancer, irrespective of the presence of underlying liver damage, appears to play the most important role.


http://www.sciencedirect.com/science/article/B7582-4C708M6-6/2/05a76fd0de3f91dd2beda51c02e69386

Background. Keratin 8 is a major component of intermediate filaments in single-layered epithelia of the gastrointestinal tract. Keratin 8 deficient mice display signs of colitis and diarrhoea characteristic for inflammatory bowel disease. Very recently, two keratin 8 mutations, Y54H and G62C, were identified. Aims. We investigated if these keratin 8 missense mutations were associated with inflammatory bowel disease. Patients. In total, 217 German patients with Crohn's disease, 131 German patients with ulcerative colitis, and 560 German control subjects were enrolled in this study. Methods. Samples were analysed by PCR amplification and subsequent melting curve analysis using fluorescence resonance energy transfer probes. Results. The G62C mutation was detected in five (2.3%) patients presenting with Crohn's disease and in three (2.3%) with ulcerative colitis. In comparison, 9 (1.6%) out of 560 controls were heterozygous for this mutation. No patient or control was homozygous for this mutation. Patients carrying one mutant allele did not show any noticeable characteristics in their corresponding phenotype. In contrast, the Y54H mutation was observed in neither any of the 348 patients with inflammatory bowel disease nor in any control subject. Conclusions. Our data indicate that both keratin 8 mutations, G62C and Y54H, do not play a relevant pathogenic role in inflammatory bowel disease.

Background. Keratin 8 (K8) and 18 (K18) are the major components of the intermediate filament cytoskeleton of pancreatic acinar cells and play a relevant role in pancreatic exocrine homeostasis. Transgenic mice for K8 have shown to display progressive exocrine pancreas alterations, including dysplasia, loss of acinar architecture, redifferentiation of acinar to ductal cells, inflammation, fibrosis, and substitution of exocrine tissue by adipose tissue. Aim. To investigate whether mutations in the keratin 8 gene are associated with chronic pancreatitis. Methods. Mutations in the keratin 8 gene were determined by polymerase chain reaction/restriction fragment length polymorphism in 67 chronic pancreatitis patients and 100 normal controls. Sequence analysis was performed when necessary. Results. Glycine-to-cysteine mutations at position 61 (G61C) of the keratin 8 gene were found in six patients (8.9 vs. 0%, pcConclusion. G61C mutation of the keratin 8 gene, together with other environmental factors and/or genetic factors, could predispose to chronic pancreatitis, by interfering with the normal organization of keratin filaments.


Background. Several studies have demonstrated that bone marrow contains a subpopulation of stem cells capable of participating in the hepatic regenerative process, even if some reports indicate quite a low level of liver repopulation by human stem cells in the normal and transiently injured liver. Aims. In order to overcome the low engraftment levels seen in previous models, we tried the direct intraperitoneal administration of human cord blood stem cells, using a model of hepatic damage induced by allyl alcohol in NOD/SCID mice. Methods. We designed a protocol based on stem cell infusion following liver damage in the absence of irradiation. Flow cytometry, histology, immunohistochemistry and RT-PCR for human hepatic markers were performed to monitor human cell engraftment. Results. Human stem cells were able to transdifferentiate into hepatocytes, to improve liver regeneration after damage and to reduce the mortality rate both in both protocols, even if with qualitative and quantitative differences in the transdifferentiation process. Conclusions. We demonstrated for the first time that the intraperitoneal administration of stem cells can guarantee a rapid liver engraftment. Moreover, the new protocol based on stem cell infusion following liver damage in the absence of irradiation may represent a step forward for the clinical application of stem cell transplantation.


Background. Exfoliated colonic epithelial cells in faeces provide a source of human DNA which may be analysed for the presence of tumour-induced modification. Aim. In the present study we
investigated K-ras and p53 mutations in faeces of patients with colorectal carcinoma, to verify whether analysis of these mutations might identify a high percentage of patients with colorectal cancer. Patients and methods. Faeces, tumour and normal mucosa samples were taken from 26 patients. Polymerase chain reaction amplification and restriction enzyme analysis were performed to detect K-ras mutations; p53 gene mutations were identified by using polymerase chain reaction amplification and single strand conformation polymorphism. Results. We were able to amplify the K-ras gene and exons 5-9 of the p53 gene in 100% of the faecal samples studied. K-ras and p53 gene mutations were detected in faeces in 26.9% and 50% of the cases, respectively. The two mutations were present together in 5 out of 26 patients. There was full agreement between the K-ras and p53 pattern observed in faecal DNA and that in tumour tissue DNA. Conclusions. Application of K-ras and p53 mutation gene analysis in the faeces may have clinical applications in the future. Since this genetic analysis is able to detect only 57.7% of patients with colorectal cancer, the study of other genes involved in colorectal carcinogenesis is necessary.


http://www.sciencedirect.com/science/article/B7582-4B1SKT2-P/2/752fac7721a1a8381722a932c03523ccc35

Background. In vitro studies showed that Helicobacter pylori strains carrying the cag pathogenicity island are able to induce epithelial secretion of Interleukin-8. Aims. To evaluate the assessment of cag pathogenicity island and the expression of Interleukin-8 in the gastric mucosa of Helicobacter pylori-infected patients and correlate these data with the activity of gastritis and Helicobacter pylori density. Methods. cag status was determined by polymerase chain reaction directly on gastric biopsies from 13 Helicobacter pylori+ patients with non-ulcer dyspepsia and 13 Helicobacter pylori+ with duodenal ulcer. Interleukin-8 gene transcription and protein expression were analysed by in situ hybridization and immunofluorescence, respectively. Gastritis activity and Helicobacter pylori density were also investigated. Results. cag was present in 20/26 of Helicobacter pylori+ patients: in 7/13 non-ulcer dyspepsia (53.8%) and in 13/13 duodenal ulcer patients (100%). (pcag+ than in cag- patients (pHelicobacter pylori density was enhanced in cag+ (pConclusions. The present study demonstrates that in Helicobacter pylori-infected human gastric mucosa, cag+ infection is associated with enhanced Interleukin-8 expression, higher levels of active gastritis and bacterial density, and presence of duodenal ulcer.


http://www.sciencedirect.com/science/article/B7582-4BY3WJ1-1/2/accf7993f832312b2d28c9298cb23ec2

Coeliac disease is an autoimmune enteropathy characterized by an enhanced permeability of the intestinal epithelial barrier. In epithelial cells paracellular permeability is regulated by intercellular tight junction. The cytoplasmic protein ZO-1 interacts directly with F-actin and plays a pivotal role in the structural and functional organization of tight junction. Aim. The aim of this study was to investigate the expression and localization of ZO-1 in the intestinal mucosa of coeliac patients. Patients and methods. Twenty patients with active coeliac disease, seven of whom underwent a repeat biopsy following a gluten-free diet and 27 control subjects, were studied. In all subjects, three biopsies were obtained from distal duodenum during upper gastrointestinal endoscopy. ZO-1 protein localization and levels were detected by immunofluorescence followed
by confocal microscopy analysis and immunoblotting. ZO-1 mRNA expression was assessed by RT-PCR. F-actin distribution was also investigated. Results. In patients with active coeliac disease, both ZO-1 protein levels and mRNA were clearly reduced. Cytoskeletal organization was disrupted with F-actin staining concentrated at the subcortical and basal surface regions. Abnormalities in ZO-1 expression and actin organization were reversed after a gluten-free diet. Conclusions. In active coeliac disease, ZO-1 protein expression is downregulated at the transcriptional level in association with F-actin redistribution. These changes are completely reversed after a gluten-free diet and could contribute to the increased intestinal paracellular permeability observed in this disorder.


http://www.sciencedirect.com/science/article/B7582-4B1SKT2-10/2/ef05617cb932667ad27399b63524a440

Background. The mammalian augmenter of liver regeneration gene encodes a protein involved in the unique process of liver regeneration. The augmenter of liver regeneration respective protein stimulates hepatocyte proliferation in hepatectomized rats and inhibits cytotoxic activity of liver-derived Natural Killer cells from intact rats. Augmenter of liver regeneration protein shares homology with a Saccaromyces Cerevisiae protein essential for the viability, oxidative phosphorylation and cell-division cycle. Aims. To demonstrate if augmenter of liver regeneration protein, like the homologous in the yeast, plays a role in the regulation of biogenesis of mitochondria. Methods. Augmenter of liver regeneration protein was injected in intact rats and, in the hepatic tissue, the expression of two genes located in two different regions of the mitochondrial genome, mitochondrial ATPase 6/8, and ND1 subunit, and of a nuclear gene, mitochondrial Transcription Factor A, were considered. In addition, cytochrome content and oxidative phosphorylation capacity of liver-derived mitochondria were evaluated. Results. The augmenter of liver regeneration protein administration induces an increase in the mitochondrial gene expression and enhances cytochrome content and oxidative phosphorylation capacity of liver-derived mitochondria. Conclusions. The present data demonstrate a comparable role in the regulation of mitochondria biogenesis in the eukaryotic cell like the yeast protein. This phenomenon could be part of the complex mechanism through which augmenter of liver regeneration regulates hepatocyte proliferation.


http://www.sciencedirect.com/science/article/B7582-4B2H630-1P/2/4a473cd7baa8504d93f7c4bc70f8b955

Background. We have shown that the administration of exogenous Augmenter of Liver Regeneration protein in intact rats i) regulates mitochondrial gene expression by inducing the transcription and translation of the nuclear-encoded mitochondrial transcription factor A, and ii) inhibits the lytic activity of liver-resident Natural Killer cells. Aims. The present investigation was carried out to study the effect, in intact rats, of exogenous administration of Augmenter of Liver Regeneration protein on Interferon-[gamma], a cytokine produced by activated Natural Killer cells and known to control the expression of mitochondrial transcription factor A, a nuclear gene responsible for mitochondrial metabolism. Methods. Interferon-[gamma] was measured as messenger RNA in liver-derived mononuclear leukocytes and as protein in liver-derived Natural
Killer cells after a single injection of Augmenter of Liver Regeneration protein. Results. The data obtained demonstrate that: i) in intact rats, Augmenter of Liver Regeneration protein administration induces a reduction of Interferon-[gamma] in the liver-resident Natural Killer cells and ii) the administration of Interferon-[gamma] in 70% hepatectomized rats is followed by a significant reduction both of the mitochondrial transcription factor A expression and of liver regeneration. Conclusions. These data demonstrate the pivotal role of Augmenter of Liver Regeneration as Growth Factor and as immunoregulator by controlling, through Interferon-[gamma] levels, the mitochondrial transcription factor A expression and the lytic activity of liver-resident Natural Killer cells.


http://www.sciencedirect.com/science/article/B7582-4B1WVB-7C/2/8591fd99e97d5d8eb29e96bf3d6fd737

Background. Host response plays a major role in pathogenesis of Helicobacter pylori-induced gastroduodenal diseases including adenocarcinoma of distal stomach. Epidermal growth factor-related growth factors are important modulators of gastric homeostasis in normal and damaged gastrointestinal mucosa. Aim. To evaluate expression of heparin binding epidermal growth factor and amphiregulin in antral mucosa of Helicobacter pylori-infected and non-infected dyspeptic patients and to correlate levels of heparin binding-epidermal growth factor and amphiregulin mRNA with mitogenic activity of gastric epithelial cells. Methods. A total of 10 Helicobacter pylori-infected and 15 Helicobacter pylori non-infected (10 with and 5 without gastritis) dyspeptic patients were studied. Diagnosis of Helicobacter pylori infection was based on rapid urease test and histology. Heparin binding-epidermal growth factor and amphiregulin mRNA expression in antral mucosa were assessed by reverse transcriptase-polymerase chain reaction. Protein expression and localization of both peptides were determined by immunohistochemistry. Mitogenic activity of antral gastric mucosa was assessed by determination of proliferating cell nuclear antigen labelling index by immunohistochemistry. Results. Heparin binding-epidermal growth factor and amphiregulin mRNA expression increased in Helicobacter pylori-infected vs Helicobacter pylori non-infected patients. Heparin binding-epidermal growth factor and amphiregulin immunostaining was more intense and deeper in gastric gland compartment in infected mucosa than in noninfected mucosa. Increase in heparin binding-epidermal growth factor and amphiregulin mRNA expression significantly correlated with increase in proliferating cell nuclear antigen labelling index. Conclusions. Helicobacter pylori gastritis is associated with up-regulation of heparin binding-epidermal growth factor and amphiregulin which correlates with increased mitogenic activity of gastric mucosa. Increased heparin binding-epidermal growth factor and amphiregulin expression is postulated to contribute to reparative response of gastric mucosa to Helicobacter pylori infection.

DNA Repair (15)

DNA from therapy-related acute leukemia/myelodysplastic syndrome cases (tAL/MDS) from the GIMEMA [Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto] Archive was examined for the microsatellite instability (MSI+) phenotype that is diagnostic for defective DNA mismatch repair. More than 60% (16/25) of tAL/MDS cases were MSI+ in contrast to de novo cases. hMLH1 gene silencing was rare and evidence of promoter methylation was found in less than one-third of the MSI+ cases. Among the GIMEMA patients who had been treated for breast cancer there was an apparent trend towards early onset primary breast disease. This suggests that there might be common predisposing factors for breast cancer and tAL/MDS. There were also three examples of mutations in the MRE11 gene among the 25 tAL/MDS cases suggesting that defective recombinational DNA repair may promote the development of secondary malignancy. MSI+ tAL/MDS was significantly associated with previous chemotherapy and the frequency of MSI+ among radiotherapy patients was considerably lower. In view of the established relationship between drug resistance and mismatch repair defects, we suggest that selection for therapeutic drug resistance may contribute to the incidence of MSI+ tAL/MDS.


The patterns of expression of 3 human DNA-repair genes (ERCC1, ERCC2, ERCC6) were assessed in 52 bone-marrow specimens obtained from cancer patients prepared for autologous bone-marrow transplantation. Marrow was collected prior to the initiation of treatment in patients with sarcoma or testicular cancer; marrow was collected after initial cytoreductive therapy for patients with non-Hodgkin's lymphoma, Hodgkin's disease, and other tumors. Slot-blot analysis of marrow RNA showed a bimodal pattern of ERCC1, ERCC2 and ERCC6 gene expression with relative expression values ranging more than 200-fold. This pattern was seen in all patient groups and appeared to be independent of whether or not patients had received prior chemotherapy. In all patient groups, when expression was low for ERCC1, expression was also low for ERCC2 and ERCC6, suggesting that expression of these genes may be coordinated within an individual although they are located on two different chromosomes. Southern blot analyses of Pst I digest of DNA from 6 bone-marrow samples indicate no differences in ERCC1 gene copy number between high expressors and low expressors. There is absence of restriction fragment length polymorphism for ERCC1 suggesting that the different levels of expression in high and low expressors were not due to major deletions or rearrangements of the ERCC1 gene. We conclude that expression of these ERCC genes may vary widely between individuals, and that within an individual, their expression may be linked and coordinated by a common regulatory mechanism.


A new approach to monitoring UV damage and repair in the human genome has been developed. The proposed approach is based on a combination of features unique to interspersed repetitive Alu elements, and the ability of certain DNA lesions to block Taq polymerase-mediated DNA
synthesis: namely, the extraordinary abundance of Alu repeats throughout the human genome in conjunction with distinct sequence motifs comprising long runs of T residues which are likely targets for formation of UV lesions. Hence, Taq polymerase-mediated extension synthesis with Alu specific primers was employed to visualize formation of discrete predicted adducts within the element. Several variations of the Alu-primer driven amplification protocol were developed to monitor the following aspects of damage: (i) induction of UV-photoproducts at predicted sites within the Alu sequence, (ii) modification of extension synthesis profiles, (iii) UV dose dependent, quantitative inhibition of Alu-primer driven amplification. The assays reveal sites of predicted Taq polymerase blockage within the Alu sequence, a global decrease in the mean length of extension products, and a measurable reduction in the quantity of extension products that is inversely proportional to UV dose. Thus, the exceptional abundance of Alu repeats and their primary sequence features, in combination with the ability of UV lesions to block elongation by Taq polymerase, provide a novel and sensitive system for detecting UV damage in the human genome. The system detects UV damage at levels that are compatible with cellular DNA repair, and provides a unique amplification-based protocol for probing the overall integrity of human DNA.


http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-4/2/397304a310fed992018fff4a22ce43

The Saccharomyces cerevisiae RAD52 gene was introduced into the human fibrosarcoma-derived cell line HT1080. Transfected cell lines that expressed the yeast transgene catalyzed inter-plasmid homologous DNA recombination at frequencies approx. 12-fold higher than did control cells. Additional experiments revealed that yeast RAD52 gene expression increased the level of resistance to the DNA damaging agents diepoxybutane, and methyl methanesulfonate, but did not alter sensitivity to ultraviolet radiation. These results indicate that the S. cerevisiae Rad52 protein can function in a human somatic cell background and provide support for the idea that a homologous recombination-based DNA repair process functions in mammalian somatic cells.


http://www.sciencedirect.com/science/article/B6X17-464P4Y9-1/2/8452f3886ec3c02d002e68f77e0ecd6

The gene p53 is a critical tumor suppressor that can respond to multiple signals of cellular gatekeepers for growth and division. The mdm2 gene is one of the downstream target genes for transcriptional activation by the product of p53 tumor suppressor gene. Transactivation of mdm2 gene is represented by the presence of a functional P53 protein. To understand the biological function of mutant p53 in tumorigenesis, we constructed a number of p53 mutants by site-directed mutagenesis (H179Y, L194R, S240R, R249S, A276D, E286Q), followed by characterization of each P53 mutant's ability to transactivate mdm2, bax and p21waf. The transactivation properties of p53 mutants were compared by co-transfection with pGL-3-mdm2, pGL-3-bax and pGL-3-p21waf into the P53 null cell line H1299 derived from a non-small cell lung carcinoma. Among them mt p53 S240R and E286Q were shown to have enhanced transactivating activity of pGL3-mdm2, at about 43.2 and 28.2% of the wt p53 vector, respectively, while the remaining four had nearly the same level of activity as the negative control did. Furthermore, data indicated that mt
p53 S240R had as high an ability to suppress the growth of the p53 null cell line H1299 as wild type p53. Therefore, mutant p53 alone is an insufficient indicator of poor prognosis. Instead, functional p53 may affect lung cancer prognosis.


http://www.sciencedirect.com/science/article/B6T2B-3TC13P0-8/2/e5d6486f80e4b0be5a4cbca280992857

In previously reported studies, we transfected repair-proficient murine fibroblasts with the denV gene of bacteriophage T4 and showed that expression of encoded endonuclease V markedly enhanced cyclobutane pyrimidine dimer (CPD) repair and reduced the frequency of ultraviolet radiation (UV)-induced mutations. In the present studies, we compared the spectra of UV-induced mutations at the hprt locus in denV-transfected and control cells. A significant difference in mutation types was observed. While multiple base deletions and single base insertions were found in denV-transfected but not control cells, multiple tandem and non-tandem point mutations identified in control cells were absent in denV-transfected cells. When we compared colony survival following UV exposure in the two cell lines, it appeared that endonuclease V expression did not enhance UV resistance, instead denV-transfected cells had increased susceptibility to low fluences of UV. The effects of endonuclease V expression on UV resistance and on UV mutational spectrum are likely to be due both to the removal of CPDs and to the novel enzymatic activity of endonuclease V.


http://www.sciencedirect.com/science/article/B6T2B-3TXKBPW-3/2/0c205c6100fa571e7bd065f96f9635e6

Apurinic/apyrimidinic endonuclease (here designated APE/REF) carries out repair incision at abasic or single-strand break damages in mammals. This multifunctional protein also has putative role(s) as a cysteine 'reducing factor' (REF) in cell-stress transcriptional responses. To assess the significance of APE/REF for embryonic teratogenesis we constructed a more precisely targeted Ape/Ref-deficient genotype in mice. Ape/Ref gene replacement in ES cells eliminated the potential of APE/REF protein synthesis while retaining the Ape/Ref bi-directional promoter that avoided potential inactivation of an upstream gene. Chimeric animals crossed into Tac:N:NIHS-BC produced germline transmission. Homozygous null Ape/Ref-embryos exhibited successful implantation and nearly normal developmental progression until embryonic day 7.5 followed by morphogenetic failure and adsorption of embryos by day 9.5. We characterized the cellular events proceeding to embryonic lethality and examined ionizing radiation sensitivity of pre-implantation Ape/Ref-null embryos. After intermating of heterozygotes, Mendelian numbers of putative Ape/Ref-null progeny embryos at day 6.5 displayed a several-fold elevation of pycnotic, fragmenting cell nuclei within the embryo proper--the epiblast. Increased cell-nucleus degeneration occurred within epiblast cells while mitosis continued and before obvious morphogenetic disruption. Mitogenic response to epiblast cell death, if any, was ineffective for replacement of lost cells. Extra-embryonic yolk sac, a trophectoderm derived lineage retained normal appearance to day 9. Explanted homozygous Ape/Ref-null blastocysts displayed increased sensitivity to [gamma]-irradiation, most likely a manifestation of APE/REF incision defect. Our study establishes that this new Ape/Ref deficiency genotype is definitely capable of
post-implantation developmental progression to the onset of gastrulation. Function(s) of APE/REF in base damage incision and also conceivably in mitogenic responses towards epiblast cell death are critical for transit through the gastrulation stage of embryonic growth and development.

http://www.sciencedirect.com/science/article/B6T2B-3RWW7NX-3/2/f3c154ae994d846d2624b5c912904ecd

A search for genetic alterations within the XPG gene has been conducted on skin and blood cells cultured from a newly characterized xeroderma pigmentosum (XP) patient (XP20BE). This patient is the ninth known case that falls into the extremely rare XP complementation group G. Four genetic markers within the XPG gene (including two polymorphisms) demonstrated the Mendelian distribution of this gene from the parents to the patient and to an unaffected sibling. The patient (XP20BE) inherited a G to T transversion from his father in exon 1 of the XPG gene that resulted in the conversion of a glutamic acid at codon 11 to a termination codon. The patient also inherited an XP-G allele from his mother that produces an unstable or poorly expressed message. The cause of the latter defect is still uncertain. In addition to these alterations, XP20BE cDNA contained an mRNA species with a large splicing defect that encompassed a deletion from exon 1 to exon 14. This splicing defect, however, appears to be a naturally occurring low-frequency event that results from abnormal splicing that occurs between certain conserved non-consensus splicing signals within the human XPG gene.

http://www.sciencedirect.com/science/article/B6T2B-3Y8W992-2/2/f2e26411506fc21d396e71431645045a

We have cloned a 13 kb genomic DNA fragment from the Chinese hamster ovary cell line, CHO-KI, and determined the nucleotide sequence of a 4 kb stretch of DNA which encompasses the complete sequence (2.277 kb) of the hamster apurinic/apyrimidinic endonuclease (chAPE1) gene. The intron/exon boundaries, identified by RT-PCR, follow GT/AG rule. The structure of the chAPE1 gene is similar to other mammalian apurinic/apyrimidinic (AP) endonuclease (hAPE1, BAP1, rAPEN and mAPE1) genes in that it has five exons and four introns with the first exon unexpressed. This structure, however, differs from one of the two structures that have been proposed for mAPE1 gene. Three transcription start sites (TSS) for the chAPE1 gene were identified by primer extension analysis at +1, +14 and +18 positions. The sequence also includes 1.72 kb of the upstream region of the chAPE1 gene. In this region, a CCAAT box but no TATA box that could initiate the transcription at the initiation sites was identified. The upstream region also includes the binding sites for a variety of other transcription factors. A polyadenylation signal, 13 nucleotides downstream to the polyadenylation signal, was identified by 3'-RACE analysis. The observed 1.28 kb transcript of the chAPE1 gene is smaller than the 1.5 kb transcript of the human AP endonuclease gene. The translation of chAPE1 gene starts within the second exon with ATG and terminates in the fifth exon with UGA codons, 318 and 2121 nucleotides downstream to the first TSS, respectively. The encoded peptide of 317 amino acid residues is similar in size and is highly homologous in its amino acid sequence to mouse, rat, human, and bovine AP endonucleases.

http://www.sciencedirect.com/science/article/B6T2B-3VYTVB6-4/2/3221a26e3eada48db2cfdadb9c16a986

The role of poly(ADP-ribose) polymerase (PADPRP) in nuclear DNA repair and other nuclear processes has been intensely studied and debated for decades. Recent studies have begun to shed new light on these arguments with firm experimental data for its role, primarily, as a remodeler of chromatin structure. Those studies imply that PADPRP plays an indirect role in DNA repair, serving to expose DNA to repair enzymes through chromatin remodeling. Only DNA that is tightly packaged would require PADPRP activity for its repair; while DNA in an open conformation would be accessible to DNA repair enzymes and not require PADPRP activity. The purpose of the current studies was to address the above hypothesis directly. Using quantitative Southern blot analysis, we studied repair in transcribed and nontranscribed nuclear DNA sequences in ADPRT 351 cells 95% deficient in PADPRP activity. Cells were exposed to methylnitrosourea (MNU) for 1 h and allowed to repair for 8 or 24 h. Densitometric scans of autoradiographs revealed that, when compared to their parental V79 cell line, ADPRT 351 cells 95% deficient in PADPRP activity were equally as efficient in repair of N-methylpurines in the transcribed sequence containing the dihydrofolate reductase gene. However, the ADPRT 351 cells were deficient in the ability to repair these lesions in the nontranscribed sequence containing the IgE gene compared to repair of the same sequence in the parental V79 cells. Nucleoid sedimentation assays demonstrated that the ADPRT 351 cells are deficient in repair across the entire genome when compared to the parental V79 cells. These studies indicate that PADPRP activity is not required for repair of N-methylpurines in transcribed nuclear DNA sequences but is necessary for the repair of these lesions in nontranscribed nuclear DNA sequences as well as across the entire genome since the DNA in a given cell is predominantly nontranscribed.


We have developed a rapid method to synthesize radioactively labeled single-stranded DNA probes suitable for strand-specific analysis of single copy genes on Southern blot. Linear PCR with 10 [mu]Ci [alpha]32P-aATP (3000 Ci/mmol) as the only dATP source enabled us to generate strand-specific DNA probes with high specific activity. The probes synthesized by this method have higher specific activities and the same strand specificity compared to the end-labeled single-stranded DNA probes obtained from single-stranded M13mp18/19 vectors. Application of the method for strand-specific analysis of ultraviolet-induced DNA lesions in defined DNA sequences significantly improved the hybridization signal.


http://www.sciencedirect.com/science/article/B6X17-485X6NB-
Base excision repair (BER) is a tightly coordinated mechanism for repair of DNA base damage (via alkylation and oxidation) and base loss. From E. coli to yeast to human cells, subtle alterations in expression of BER proteins lead to mutagenic or genome instability phenotypes. DNA polymerase [beta] ([beta]-pol), the major BER polymerase, has been found to be over-expressed in human tumor tissues and more recently it has been shown that over-expression of [beta]-pol results in a mutator and genome instability phenotype. These previous reports imply that [beta]-pol over-expression is deleterious and suggests that such an imbalance may cause an overall functional deficiency in the BER pathway. In the present study, we have developed a bicistronic tetracycline-responsive transgenic system to over-express [beta]-pol in mice. We find that over-expression of [beta]-pol in the lens epithelium results in the early onset of severe cortical cataract, with cataractogenesis beginning within 4 days after birth. In utero and post-natal suppression of transgenic Flag-[beta]-pol expression by doxycycline administration completely prevents cataract formation through adulthood, yet cataract is subsequently observed following removal of doxycycline and re-expression of the transgene. Cataract development accompanies increased expression of cyclooxygenase-2 in the lenticular fibers of the lens, implicating oxidative stress in the development of this cataractous phenotype. Although the mechanism for the transgene mediated cataractogenesis is not clear at this time, it is nevertheless intriguing that increased expression of [beta]-pol leads to such a phenotype. These results suggest that either a [beta]-pol expression imbalance negatively affects overall fidelity and/or BER capacity or that [beta]-pol has a role in lens epithelial cell differentiation.


http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-3/2/c2a0dcd4caf5235480e1c448c5f59fb4

XP12BE is a commonly studied XP-A cell line that exhibits slightly increased resistance to UV compared with the majority of XP-A cell lines. The elevated UV survival is common to a subset of XP-A cell lines and correlates with delayed onset of the neurological disease in patients. We identified the XPA mutations in XP12BE by single strand conformation polymorphism (SSCP) analyses and nucleotide sequencing. XP12BE is a compound heterozygote and both mutations affect mRNA splicing. One mutation is a G to C transversion within the splice donor site of intron 4 that is common to several cell lines from XP-A patients with delayed onset of neurological disease. The other mutation is a G to T transversion at the same position as a G to C transversion in the splice acceptor site of intron 3 that is common in Japanese XP-A patients. We also demonstrated the persistence of the XP12BE mutations in cell line 2-O-A2 which has been shown to express XPA protein. These results suggest that the intron 4 splice donor mutation likely produces some, at least partially functional, XPA protein that accounts for the increased UV survival of XP-A cell lines derived from patients with delayed onset of neurological disease.


http://www.sciencedirect.com/science/article/B6T2B-3VXYRFN-4/2/74d7dec1b7157c4f199ca06b0074c24c

The REV3 gene of Saccharomyces cerevisiae encodes the catalytic subunit of DNA polymerase
[zeta] which is involved in translesion synthesis. The mouse homolog of this gene, Rev3l, was cloned and sequenced. The gene encodes a putative protein of 3122 amino acids. The sequence conservation to its yeast counterpart is restricted to several regions. In the carboxy-terminal part of the protein all six domains are present that are characteristic for [alpha]-type DNA polymerases. In the amino-terminal part of the protein two regions can be identified with considerable similarity to the NT boxes of mouse polymerase [delta]. In addition, a region of 60 residues unique for the REV3 homologs can be found in the middle part of the protein. The mouse REV3L protein shows strong sequence conservation with the recently cloned human REV3L protein (86% identity overall). Northern blot analysis of various tissues of the mouse revealed that transcription of the Rev3l gene was highest in brain, ovaries and testis. The human REV3L gene was localised to the long arm of chromosome 6, region 21-22. The mouse equivalent maps to chromosome 10, distal to the c-mycb gene, close to the Macs gene.


http://www.sciencedirect.com/science/article/B6T2B-3WD5C3J-1/2/703efb76af1fbb8b03f37c1b178dfca33

We report a sensitive, SINE (Short Interspersed DNA Element)-mediated, PCR-based, DNA damage detection assay. Here, the SINE assay is used for detection of UVB-induced DNA damage and repair in cultured mouse cells and in vivo, in mouse skin. The unique feature of the SINE assay is its ability to support simultaneous amplification of multiple, random segments of genomic DNA. This can be accomplished due to the remarkable abundance, dispersion and conservation of SINEs in mammalian genomes. The most abundant SINEs in the mouse genome are the B1 elements, at a copy number of 50,000-80,000. Due to their strong sequence conservation, primers complementary to the B1 consensus sequence anneal to the majority of their targets in the genome. Consequently, long segments of genomic DNA located between pairs of B1 elements are efficiently amplified by PCR. Thus, in conjunction with the fact that many types of DNA adducts form blocks for thermostable polymerase, the B1 element anchored PCR makes a sensitive and versatile tool for assessing the overall integrity of the transcribed regions in mouse genome. We measured UVB-dose (0.1-3 kJ m-2) dependent formation of photoproducts in DNA from cultured cells, and after 20 h observed a substantial removal of damage at doses lower or equal to 0.6 kJ m-2. The sensitivity of detection of UVB-photoproducts formation and repair was compared to that of the conventional, single locus-targeting QPCR. Using the SINE assay we also have shown the distribution of UVB and UVC induced DNA adducts at a single nucleotide resolution within the B1 elements in mouse DNA. Lastly, we demonstrated that the sensitivity of the SINE assay is adequate for measurement of UVB-dose (1-6 kJ m-2) dependent formation and subsequent removal of photoproducts in vivo, in mouse skin.

**Domestic Animal Endocrinology** (4)

Cordano, P., H. M. Hammon, et al. (2000). "mRNA of insulin-like growth factor (IGF) quantification and presence of IGF binding proteins, and receptors for growth hormone, IGF-I and insulin, determined by reverse transcribed polymerase chain reaction, in the liver of growing and mature
Plasma insulin-like growth factor-I (IGF-I) concentrations were related to hepatic levels of IGF-I mRNA measured by competitive reverse transcription polymerase chain reaction (RT-PCR) (RT-PCR) in neonatal (8 d old) calves, veal calves, fattened castrated bulls and mature intact bulls. Furthermore, the presence of mRNAs of IGF-II and of receptors for IGF-I (IGF-IR), growth hormone (GHR) and insulin (IR), as well as mRNAs of IGF binding proteins (IGFBP-1, -2 and -3) were assessed by RT-PCR. Hepatic IGF-I mRNA levels and plasma IGF-I concentrations in veal calves, fattened castrated bulls and in intact bulls were 4 to 8 times higher than in 8-d old calves and were 2 to 3 times higher in calves fed colostrum than in calves fed only milk replacer. Hepatic IGF-I mRNA concentrations were closely correlated (r = 0.92) with plasma IGF-I concentrations, suggesting that hepatic IGF-I production largely determines plasma IGF-I levels. The presence of IGF II, IGF-IR, GHR, IR and IGFBP-1, -2 and -3 mRNA was confirmed in the liver of 8-d old calves and older cattle as well, and among newborn calves their presence was independent of differences in nutrition. In conclusion, the major hepatic components of the GH-IGF axis were present in neonatal calves, but the IGF-I expression and therefore also plasma IGF-I levels were relatively low.


The amino acid sequence of bovine somatotropin (bST) varies at position 127 where either valine or leucine is found. The frequencies of leucine127 and valine127 bST gene alleles in cows (n = 302) and sires (n = 70) from major dairy breeds (Holstein, Brown Swiss, Guernsey, Jersey, and Ayrshire) were determined using DNA extracted from whole blood or spermatozoa. A 428 base pair fragment of the bST gene was amplified using polymerase chain reaction (PCR) and variants of the bST gene were detected as polymorphisms by Alu I restriction endonuclease digestion of PCR products. Restriction enzyme DNA fragments for the leucine127 variant were 265, 96, 51, and 16 base pair and for the valine127 variant were 265, 147, and 16 base pair as a polymorphism of bST was present in the 147 base pair DNA fragment. Frequencies of leucine127 and valine127 alleles for cows (n=302) were 1.0 and 0 for Brown Swiss, 0.93 and 0.07 for Holstein, 0.79 and 0.21 for Ayrshire, and 0.56 and 0.44 for Jersey, respectively. In Holstein sires used for artificial insemination (n=70), the frequency of leucine127 and valine127 alleles was 0.96 and 0.04. Estimates of transmitting ability for milk production tended to be greater for Holstein cows that were homozygous for leucine127 bST and Jersey cows that were homozygous for valine127 bST whereas Holstein sires with different bST genotypes were similar. In summary, frequencies of alleles for the bST gene were not similar in different dairy breeds and estimates of milk production were correlated with bST gene variant in cows but not sires.

Objectives were to establish conditions for preparation, growth, and maintenance of a primary culture cell model of fetal uterine cells, and to determine whether cells maintained under those conditions would maintain their capacity to respond to estrogen stimulation in vitro. Fetal uteri (n = 19) were enzymatically dispersed and grown on Type 1 collagen in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum. Fetal-uterine cells appeared fibroblast-like and exhibited positive immunostaining for both vimentin and cytokeratin. Effects of gestational age (GA), passage number (p), and hormonal treatment on appearance of specific mRNAs were determined by RT-PCR; relative concentrations of products determined by densitometry were analyzed as the ratio of target cDNA to the GAPDH loading control. Cells expressed mRNAs for estrogen receptor (ER), TGF-[beta], EGF-R, PRL-R, IL-1 [alpha], and IL-6. ER mRNA was greater at 185-200 than at 100-110 d GA (P < 0.01). All specific mRNAs examined were greater in p5 cells than p2 at both 100-110 (P < 0.01) and 185-200 d GA (P < 0.02). There was no effect of estradiol on these specific mRNAs in cells from 100-110 d GA; at 185-200 d GA, there was an estradiol (1.0 n) effect both at 6 hr (P < 0.001) and 24 hr (P < 0.02). Overall, there was an effect of 8-br-cAMP (1 m; 6 h) on specific mRNAs in cells at both 100-110 (P < 0.001) and 185-200 d GA (P < 0.001). In p5 cells from Day 185-200 GA, there was increased cell proliferation (P < 0.001) in response to estradiol (1 n; 24 hr). These data suggest that primary fetal uterine cells retain their age-specific and hormone-responsive phenotype under these in vitro conditions.


http://www.sciencedirect.com/science/article/B6T62-4B6TVHD-5/2/b8332e2e585b5579dd5e277481c615a6

The effects of estradiol, insulin, and gonadotropins on levels of insulin-like growth factor binding protein (IGFBP)-2, -3, -4, and -5 mRNA levels in bovine granulosa and theca cells were evaluated in vitro using serum-free medium containing various hormone treatments arranged in four different experiments. Amounts of IGFBP-2, -3, -4 and -5 mRNA were quantitated using fluorescent quantitative real-time RT-PCR. In small-follicle (1-5 mm) granulosa cells, follicle-stimulating hormone (FSH) in the presence or absence of insulin increased (PPP7.9 mm) granulosa cells, insulin alone increased (PP>0.10). Estradiol (3 and 300 ng/ml) decreased (PPP>0.10) on IGFBP-2, -3, or -5 mRNA levels. Estradiol decreased (P<0.05) IGFBP-2, -3, and -4 mRNA levels but had no effect on IGFBP-5 mRNA levels in theca cells. LH had no effect on levels of IGFBP-2, -3, -4, or -5 mRNA in theca cells. These results indicate that expression of IGFBP-2, -3, -4, and -5 mRNA by granulosa and theca cells are differentially regulated by estradiol, insulin and gonadotropins, therefore discretely modulating the amount of bioavailable IGFs to these cells depending upon the specific hormonal stimuli. In particular, these studies are the first in cattle to show that estradiol selectively inhibits IGFBP-2, -3, and -4 gene expression in theca cells, inhibits IGFBP-5 gene expression in large-follicle granulosa cells, and stimulates IGFBP-2 gene expression in small-follicle granulosa cells.

Drug and Alcohol Dependence (1)

The D2 dopamine receptor gene (DRD2) displays Taq I restriction fragment length polymorphisms (RFLPs) at two different loci, termed A and B. One of the three different Taq I A 'alleles' described at this site, the A1 allele (size = 6.6 kb), has been found to be associated with alcoholism and with drug abuse in the majority of studies reported to date. A complete map of the Taq I A RFLP site has been constructed, through hybridization with different fragments of the 3' flanking region and polymerase chain reaction (PCR). When screening 432 unrelated individuals to establish possible A1 allelic association with drug abuse or dependence, we have encountered a novel Taq I A RFLP, which we have named 'A4' (size = 8.6 kb). This sequence variant displays a frequency of approximately 1% in our sample and shows a Mendelizing genetic pattern in an Italian nuclear family. Primers suitable for detecting A4 using PCR have been designed. The A4, but not the A3 'allele', displays substantial overlap with the A1. In particular, A2 and A3 share the presence of a Taq I restriction site, whose absence in A1 and A4 is apparently associated with substance abuse vulnerability. Therefore, in association studies it is proper to contrast individuals displaying A1 and A4 RFLP patterns, with individuals displaying A2 and A3 RFLPs.

Drug Metab. Dispos.  (9)


http://dmd.aspetjournals.org/cgi/content/abstract/32/12/1341

Cytochrome P450 2B6 (CYP2B6) metabolizes a number of therapeutic drugs and its metabolic activity varies markedly in human liver. Although genetic polymorphisms of CYP2B6 have been reported in noncoding and coding regions, little information is available regarding single nucleotide polymorphisms (SNPs) and their haplotypes in noncoding regions in Asians. Fourteen previously reported SNPs were determined by polymerase chain reaction-restriction fragment length polymorphism or SNaPshot analysis in a Korean population and their haplotypes were inferred from genotype data using an expectation-maximization algorithm. The most common haplotypes were haplotype I, the reference sequence (frequency 0.35), haplotype II (0.19), haplotype III (0.19), and haplotype V (0.12), which together accounted for 85% of all haplotypes. The frequency of haplotype III, which contains -2320C, -1778G, -1186G, -750C, and 15582T, was found to be 2.4-fold higher than that of the *1J allele in Caucasians, and the frequency of haplotype V, which contains -8207C, -1456C, -750C, 516T, and 785G, was 55% of that of the *6B allele in Caucasians. Moreover, haplotype V, the *6B allele, appeared to be completely linked to -8207 within a putative nuclear receptor binding motif, suggesting that lower expressions of the *6B allele may be associated with the presence of noncoding SNPs such as -8207G>C linked to nonsynonymous SNPs. In conclusion, we found 11 previously described polymorphisms and identified four major haplotypes of CYP2B6 in Koreans. The frequencies of the *1J or *6B alleles, which may reduce CYP2B6 enzyme expression, were found to be significantly different between Koreans and Caucasians.

In contrast to the beneficial effects of tert-butylhydroquinone (tBHQ) as a food antioxidant, a number of studies have shown that chronic exposure to tBHQ may induce carcinogenicity. Therefore, we examined the ability of tBHQ to induce the cytochrome P450 1a1 (Cyp1a1), an enzyme known to play an important role in the chemical activation of xenobiotics to carcinogenic derivatives. A significant concentration-dependent increase in Cyp1a1 mRNA, protein, and activity occurred after treatment of murine hepatoma Hepa 1c1c7 cells with tBHQ. The increase in mRNA was apparent 3 h after treatment. The RNA polymerase inhibitor, actinomycin D, completely blocked the Cyp1a1 induction by tBHQ, indicating a requirement of de novo RNA synthesis through transcriptional activation. The protein synthesis inhibitor cycloheximide superinduced the tBHQ-mediated induction of Cyp1a1 mRNA and completely prevented the increase in Cyp1a1 activity, indicating that the induction of enzyme activity by tBHQ is dependent on de novo protein synthesis. In addition, the aryl hydrocarbon receptor (AHR) antagonist, resveratrol, inhibited the increase in Cyp1a1 activity by tBHQ. Gel electrophoretic mobility shift assays showed that tBHQ causes activation or transformation of the AHR in nuclear extracts, indicating that AHR-dependent mechanisms contributed to the Cyp1a1 induction. Similar to murine Hepa 1c1c7 cells, tBHQ caused a concentration-dependent increase in CYP1A1 at the mRNA and activity levels in human HepG2 cells. This is the first demonstration that the phenolic antioxidant, tBHQ, can directly induce Cyp1a1 gene expression in an AHR-dependent manner and may represent a novel mechanism by which tBHQ promotes carcinogenicity.


http://dmd.aspetjournals.org/cgi/content/abstract/31/5/548

Human cytochrome P450 3A4 (CYP3A4) is the most abundant hepatic and intestinal phase I drug-metabolizing enzyme, and participates in the oxidative metabolism of approximately 50% of drugs on the market. In the present study, a transgenic-CYP3A4 (Tg-CYP3A4) mouse model that expresses CYP3A4 in the intestine and is phenotypically normal was generated, which was genotyped by both polymerase chain reaction and Southern blotting. Intestinal microsomes prepared from Tg-CYP3A4 mice metabolized midazolam (MDZ) to 1'-hydroxymidazolam about 2 times, and to 4-hydroxymidazolam around 3 times faster than that from wild-type (WT) mice. These increased MDZ hydroxylation activities were completely inhibited by an anti-CYP3A4 monoclonal antibody. The time course of plasma MDZ and its metabolite concentrations was measured after intravenous (0.25 mg/kg) and oral (2.5 mg/kg) administration of MDZ, and pharmacokinetic parameters were estimated by fitting to a noncompartmental model. Pretreatment with ketoconazole increased orally dosed MDZ maximum plasma concentration (Cmax), time of the maximum concentration, area under the plasma concentration-time curve from zero to infinity (AUC0-[infinity]), and elimination half-life (t1/2) to 3.2-, 1.7-, 7.7-, 2-fold, and decreased MDZ apparent oral clearance about 8-fold in Tg-CYP3A4 mice. The ratios of MDZ Cmax, AUC0-[infinity], t1/2 and bioavailability between Tg-CYP3A4 and WT mice after the oral dose of MDZ were 0.3, 0.6, 0.5, and 0.5, respectively. These results suggest that this Tg-CYP3A4 mouse would be an appropriate in vivo animal model for the evaluation of human intestine CYP3A4 metabolism of drug candidates and potential food-drug and drug-drug interactions in preclinical drug development.

In this case report, we present genetic differences in two morphine-related gene sequences, UDP-glucuronosyltransferase 2B7 (UGT2B7) and (micro) opioid receptors (MOR1), in two cancer patients whose clinical responses to morphine were very different [i.e., sensitive (patient 1) and low responder (patient 2)]. In addition, allelic variants in the UGT2B7 gene were analyzed in 46 Japanese individuals. Amplified DNA fragments for the two genes of interest were screened using single strand conformation polymorphism and then sequenced. In the UGT2B7 gene, 12 single nucleotide polymorphisms (SNPs) were newly identified with an allelic frequency ranging from 0.022 to 0.978. Six SNPs in the promoter region (A-1302G, T-1295C, T-1111C, G-899A, A-327G, and T-125C) and two coding SNPs (UGT2B7*2 in exon 2 and C1059G in exon 4) appeared to be consistently linked. Remarkable differences in the nucleotide sequence of UGT2B7 were observed between the two patients; in contrast to patient 1 who had "reference" alleles at almost SNP positions, but a rare ATTGAT*2(AT)C haplotype as homozygosity, patient 2 was a homozygous carrier for the predominant GCCAGC*1(TC)G sequence. Serum morphine and two glucuronide concentrations in patient 2 suggest that the predominant GCCAGC*1G sequence was not associated with a "poor metabolizer" phenotype. In the MOR1 gene, patient 1 had no SNPs, whereas patient 2 was a heterozygous carrier for both the G-1784A and A118G alleles. The present study describes substantial differences in genotype patterns of two genes of interest between the two patients. The results necessitate larger trials to confirm these observations in larger case control studies.


The aim of the present study was to assess the contribution of polymorphisms in the breast cancer resistance protein/ATP-binding cassette transporter G2 (BCRP/ABCG2) gene to the placental expression from a new perspective, allelic imbalance. Polymorphisms were screened by polymerase chain reaction (PCR)-single-strand conformation polymorphism analysis followed by sequencing with DNA extracted from 100 placentas. To examine whether polymorphisms of the BCRP gene correlate with the placental BCRP expression, we determined mRNA and protein levels by quantitative real-time PCR and Western blotting, respectively. In placentas, G34A (Val12Met) and C421A (Gln141Lys) were frequently observed (18-36%), but C376T, which creates a stop codon (Gln126 stop codon), was found with an allelic frequency of 1%. The mean of the BCRP protein level was significantly lower (p < 0.05) in homozygotes for the A421 allele than in those for the C421 allele, and heterozygotes had an intermediate value. To evaluate whether the C421A polymorphism acts as a cis-element in BCRP transcription, allelic imbalance was determined using informative lymphoblasts and 56 samples of placental cDNA. In most of the placental samples we tested, the difference in expression levels between the two alleles was small, and only two samples indicated a monoallelic expression (i.e., preferential expression of one allele). These results suggest that 1) the predominant allelic expression pattern of BCRP in placental samples is biallelic, and 2) the mutation C421A is not a genetic variant acting in cis, but is considered to influence the translation efficiency.

Constitutive active (or androstane) receptor (CAR, NR1I3), a member of the nuclear receptor family, is a major regulator for induction of cytochrome P450 2B (CYP2B) genes by phenobarbital. Phenobarbital-like inducer, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), is a potent mouse CAR (mCAR) ligand that has been used to study CAR target genes in mice but does not activate human CAR (hCAR) or rat CAR (rCAR). Although 6-(4-chlorophenyl) imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) was reported to be an hCAR agonistic ligand, activation of hCAR by CITCO in cell-based reporter assay was weak. Therefore, we performed a screening of 50 drugs and chemicals using cell-based reporter assays to identify activators of hCAR. Among them, HMG-CoA reductase inhibitors (cerivastatin, simvastatin, fluvastatin and atorvastatin) enhanced the hCAR-mediated transcriptional activation of PBREM reporter gene by up to 3 fold. Similar activation by HMG-CoA reductase inhibitors was also observed with mouse and rat CARs. On the other hand, pravastatin did not activate hCAR at the concentrations tested (up to 30 {micro}M). The extent of activation by the HMG-CoA reductase inhibitors was stronger than that by CITCO. Cerivastatin, simvastatin, fluvastatin and atorvastatin induced CYP2B6 mRNA in stable hCAR-expressed FLC7 cells but not in original FLC7 cells. Therefore, we concluded that CAR mediates the effects of HMG-CoA reductase inhibitors on the induction of CYP2B genes, although HMG-CoA reductase inhibitors also activate pregnane X receptor. HMG-CoA reductase inhibitors such as cerivastatin would be useful to study for elucidating molecular and cellular mechanisms of hCAR.


http://dmd.aspetjournals.org/cgi/content/abstract/33/2/254

Human carbonyl reductase (CBR) activity accounts for a significant fraction of the metabolism of endogenous and xenobiotic carbonyl compounds. It is possible that genetic polymorphisms in CBR1 and CBR3 are key for the wide interindividual variability in the disposition of CBR drug substrates. We pinpointed a single nucleotide polymorphism in CBR3 (CBR3 V244M) that encodes for a V244 to M244 change. Blacks showed a higher frequency of the M244 allele (q = 0.51, n = 49) than did whites (q = 0.31, n = 70; p = 0.003). In addition, DNA variation panels from 10 ethnic groups presented a wide range of CBR3 V244M genotype distributions. Kinetic experiments with the recombinant CBR3 protein variants and menadione revealed that CBR3 M244 has significantly higher Vmax than does CBR3 V244 (Vmax CBR3 M244 = 40.6 {+/-} 1.3 {micro}mol/min {middle dot} mg versus Vmax CBR3 V244 = 19.6 {+/-} 2.0 {micro}mol/min {middle dot} mg, p = 0.002). In contrast, both isoforms presented similar Km values (Km CBR3 M244 = 22.9 {+/-} 2.9 {micro}M versus Km CBR3 V244 = 24.6 {+/-} 3.2 {micro}M, p = 0.43). Assays with NADP(H) demonstrated a higher VmaxNADP(H) (1.6-fold) and increased catalytic efficiency (VmaxNADP(H)/KmNADP(H)) for CBR3 M244 compared with CBR3 V244 (p = 0.013). Comparative three-dimensional analyses based on the structure of the homologous porcine carbonyl reductase suggested that the V244M substitution is positioned in a region critical for interactions with the NADP(H) cofactor. These studies demonstrate that the common CBR3 V244M polymorphism encodes for CBR3 isoforms with distinctive enzymatic properties.


http://dmd.aspetjournals.org/cgi/content/abstract/30/1/4

The expression levels of mRNAs for MDR1 (P-glycoprotein), multidrug resistance-associated
proteins (MRP1, MRP2), and cytochrome P450 3A (CYP3A) in Caco-2 cells were quantitatively compared with those in human duodenal enterocytes, normal colorectal tissues, and colorectal adenocarcinomas. Caco-2 cells (passages 36-88) were kindly supplied by several laboratories in Japan. Human duodenal enterocytes were obtained from five healthy male volunteers. Normal colorectal tissues and colorectal adenocarcinomas were simultaneously obtained from seven patients with primary colorectal adenocarcinoma. MDR1, MRP1, MRP2, and CYP3A mRNA levels were determined by real-time quantitative polymerase chain reactions (PCR). Relative concentrations of mRNAs for target proteins (MDR1, MRP1, MRP2, and CYP3A) and glyceraldehyde-3-phosphate dehydrogenase in Caco-2 cells were 1.00 ± 0.15, 1.02 ± 0.06, 0.94 ± 0.10, and 0.68 ± 0.60, respectively, and those in human enterocytes were about 12-, 3-, 7-, and 8000-fold higher than in the Caco-2 cells, respectively. In contrast, MDR1, MRP1, and CYP3A mRNA levels in Caco-2 cells were comparable to those in normal colorectal tissue and colorectal adenocarcinoma.


http://dmd.aspetjournals.org/cgi/content/abstract/32/9/993

Etoposide is a DNA topoisomerase II inhibitor widely used in the treatment of a variety of malignancies that is also associated with therapy-related leukemia. The cytochrome P450 (P450)-derived catechol and quinone metabolites of etoposide may be important in the damage to the MLL (mixed lineage leukemia) gene and other genes resulting in leukemia-associated chromosomal translocations. Kinetic analysis of catechol formation by recombinant P450s was determined using liquid chromatography/selected reaction monitoring/mass spectrometry. CYP3A4 was found to play a major role in etoposide metabolism (Km = 77.7 ± 27.8 {micro}M; Vmax = 314 ± 84 pmol of catechol/min/nmol of P450). However, CYP3A5 (Km = 13.9 ± 3.1 {micro}M; Vmax = 19.4 ± 0.4 pmol of catechol/min/nmol of P450) may be involved in etoposide metabolism at therapeutic concentrations of free drug. Other P450s do not appear to be involved in etoposide catechol formation. Real-time polymerase chain reaction and Western blot analysis revealed significantly increased CYP3A4 mRNA and protein levels in hepatocytes treated with 10 {micro}M rifampicin compared with untreated cells, but only modest effects of rifampicin on CYP3A5 induction. Etoposide (40, 5, 1, and 0.25 {micro}M) caused a slight increase in CYP3A4 mRNA in three of five batches of hepatocytes but did not result in proportionately increased CYP3A4 protein levels. At high concentrations, etoposide induced only a modest increase in CYP3A5 mRNA and protein levels in four of five batches of hepatocytes. Alternatively, coadministration of other drugs with etoposide may account for the increase in etoposide catechol formation during therapy with etoposide.

Electrochimica Acta  (1)


http://www.sciencedirect.com/science/article/B6TG0-3VV9N09-P/2/9fd72598711cd8434da30e3c54a3c28e
Fully-automated detection system for manipulation of bloom-forming genera of cyanobacteria, Microcystis spp., was developed using a specific DNA probe designed based on sequence polymorphism within the 16S ribosomal DNA (rDNA) of this strain. Specific DNA sequences in 16S rDNA for Microcystis spp were determined by sequence data analysis and two probes were designed for detection of Microcystis spp. Microcystis 16S rDNA was amplified by PCR and applied to the detection system where target DNA was selectively recognized by species-specific hybridization using two DNA probes, a DNA probe conjugated on magnetic particles and a digoxigenin-labeled DNA probe. Using alkaline phosphatase labeled anti-digoxigenin antibody probe, target DNA can be detected by luminescence. In this detection method, the difference in only a few base pairs of target DNA can be discriminated and specific detection of Microcystis spp. was achieved. The appropriate annealing temperature was 60[deg]C. A computer controlled fully-automated system was used for detection of Microcystis spp and detection efficiency equivalent to that of a manual approach was obtained.

EMBO J. (9)

http://embojournal.npgjournals.com/cgi/content/abstract/22/23/6299

The human AP-endonuclease (APE1/Ref-1), a multifunctional protein central to repairing abasic sites and single-strand breaks in DNA, also plays a role in transcriptional regulation. Besides activating some transcription factors, APE1 is directly involved in Ca2+-dependent downregulation of parathyroid hormone (PTH) expression by binding to negative calcium response elements (nCaREs) present in the PTH promoter. Here we show that APE1 is acetylated both in vivo and in vitro by the transcriptional co-activator p300 which is activated by Ca2+. Acetylation at Lys6 or Lys7 enhances binding of APE1 to nCaRE. APE1 stably interacts with class I histone deacetylases (HDACs) in vivo. An increase in extracellular calcium enhances the level of acetylated APE1 which acts as a repressor for the PTH promoter. Moreover, chromatin immunoprecipitation (ChIP) assay revealed that acetylation of APE1 enhanced binding of the APE1-HDACs complex to the PTH promoter. These results indicate that acetylation of APE1 plays an important role in this key repair protein's action in transcriptional regulation.

http://embojournal.npgjournals.com/cgi/content/abstract/21/21/5899

L1 elements are ubiquitous human transposons that replicate via an RNA intermediate. We have reconstituted the initial stages of L1 element transposition in vitro. The reaction requires only the L1 ORF2 protein, L1 3' RNA, a target DNA and appropriate buffer components. We detect branched molecules consisting of junctions between transposon 3' end cDNA and the target DNA, resulting from priming at a nick in the target DNA. 5' junctions of transposon cDNA and target DNA are also observed. The nicking and reverse transcription steps in the reaction can be uncoupled, as priming at pre-existing nicks and even double-strand breaks can occur. We find evidence for specific positioning of the L1 RNA with the ORF2 protein, probably mediated in part...
by the polyadenosine portion of L1 RNA. Polyguanosine, similar to a conserved region of the L1 3' UTR, potently inhibits L1 endonuclease (L1 EN) activity. L1 EN activity is also repressed in the context of the full-length ORF2 protein, but it and a second cryptic nuclease activity are released by ORF2p proteolysis. Additionally, heterologous RNA species such as Alu element RNA and L1 transcripts with 3' extensions are substrates for the reaction.


http://embojournal.npgjournals.com/cgi/content/abstract/21/3/470

We report that the cyclophilin USA-CyP is part of distinct complexes with two spliceosomal proteins and is involved in both steps of pre-mRNA splicing. The splicing factors hPrp18 and hPrp4 have a short region of homology that defines a high affinity binding site for USA-CyP in each protein. USA-CyP forms separate, stable complexes with hPrp18 and hPrp4 in which the active site of the cyclophilin is exposed. The cyclophilin inhibitor cyclosporin A slows pre-mRNA splicing in vitro, and we show that its inhibition of the second step of splicing is caused by blocking the action of USA-CyP within its complex with hPrp18. Cyclosporin A also slows splicing in vivo, and we show that this slowing results specifically from inhibition of USA-CyP. Our results lead to a model in which USA-CyP is carried into the spliceosome in complexes with hPrp4 and hPrp18, and USA-CyP acts during splicing within these complexes. These results provide an example of the function of a cyclophilin in a complex process and provide insight into the mechanisms of action of cyclophilins.


http://embojournal.npgjournals.com/cgi/content/abstract/23/1/120

The detection of thousands of volatile odorants is mediated by several hundreds of different G protein-coupled olfactory receptors (ORs). The main strategy in encoding odorant identities is a combinatorial receptor code scheme in that different odorants are recognized by different sets of ORs. Despite increasing information on agonist-OR combinations, little is known about the antagonism of ORs in the mammalian olfactory system. Here we show that odorants inhibit odorant responses of OR(s), evidence of antagonism between odorants at the receptor level. The antagonism was demonstrated in a heterologous OR-expression system and in single olfactory neurons that expressed a given OR, and was also visualized at the level of the olfactory epithelium. Dual functions of odorants as an agonist and an antagonist to ORs indicate a new aspect in the receptor code determination for odorant mixtures that often give rise to novel perceptual qualities that are not present in each component. The current study also provides insight into strategies to modulate perceived odorant quality.


http://embojournal.npgjournals.com/cgi/content/abstract/21/6/1497

Since the discovery of RNA recombination in polioviruses, there has been a general belief that this mechanism operates only in positive-sense RNA viruses. Recently, studying wild-type Tula
hantavirus, we observed a mosaic-like structure of the S RNA segment that was consistent with
generation by recombination between viruses from two genetic lineages. Here we show
transfection-mediated rescue of Tula virus carrying recombinant S RNA segment. Independent
attempts yielded S RNA molecules of similar structure; the majority of them carried a break point
located close to one of the break points suggested for natural recombinants. Recombinant virus
purified from the original variant was able to grow to the same titers in cell culture and showed the
same characteristic immunofluorescence pattern when stained for the nucleocapsid protein.
While competent, the recombinant virus appeared to be slightly less competitive than the wild
type. Sequence analysis of the S cDNA clones obtained from the purified recombinant virus
confirmed that all S RNA molecules were of recombinant origin. This provides the first example of
a negative-sense RNA virus constructed using homologous recombination.

Scarselli, E., H. Ansuini, et al. (2002). "The human scavenger receptor class B type I is a novel candidate

http://embojournal.npgjournals.com/cgi/content/abstract/21/19/5017

We discovered that the hepatitis C virus (HCV) envelope glycoprotein E2 binds to human
hepatoma cell lines independently of the previously proposed HCV receptor CD81. Comparative
binding studies using recombinant E2 from the most prevalent 1a and 1b genotypes revealed that
E2 recognition by hepatoma cells is independent from the viral isolate, while E2-CD81 interaction
is isolate specific. Binding of soluble E2 to human hepatoma cells was impaired by deletion of the
hypervariable region 1 (HVR1), but the wild-type phenotype was recovered by introducing a
compensatory mutation reported previously to rescue infectivity of an HVR1-deleted HCV
infectious clone. We have identified the receptor responsible for E2 binding to human hepatic
cells as the human scavenger receptor class B type I (SR-BI). E2-SR-BI interaction is very
selective since neither mouse SR-BI nor the closely related human scavenger receptor CD36,
were able to bind E2. Finally, E2 recognition by SR-BI was competed out in an isolate-specific
manner both on the hepatoma cell line and on the human SR-BI-transfected cell line by an anti-
HVR1 monoclonal antibody.


http://embojournal.npgjournals.com/cgi/content/abstract/21/13/3434

We recently described an erythroid (epsilon)-globin gene repressor activity, which we named
DRED (direct repeat erythroid-definitive). We show that DRED binds with high affinity to DR1
sites in the human embryonic (epsilon-) and fetal (gamma-) globin gene promoters, but the
adult (beta)-globin promoter has no DR1 element. DRED is a 540 kDa complex; sequence
determination showed that it contains the nuclear orphan receptors TR2 and TR4. TR2 and TR4
form a heterodimer that binds to the (epsilon) and (gamma) promoter DR1 sites. One mutation in
a DR1 site causes elevated (gamma)-globin transcription in human HPFH (hereditary persistence
of fetal hemoglobin) syndrome, and we show that this mutation reduces TR2/TR4 binding in vitro.
The two receptor mRNAs are expressed at all stages of murine and human erythropoiesis; their
forced transgenic expression reduces endogenous embryonic (epsilon)-globin transcription.
These data suggest that TR2/TR4 forms the core of a larger DRED complex that represses
embryonic and fetal globin transcription in definitive erythroid cells, and therefore that inhibition of
its activity might be an attractive intervention point for treating sickle cell anemia.
Here we take advantage of the well-characterized and simple nervous system of Caenorhabditis elegans to further our understanding of the functions of RNA editing. We describe the two C.elegans ADAR genes, adr-1 and adr-2, and characterize strains containing homozygous deletions in each, or both, of these genes. We find that adr-1 is expressed in most, if not all, cells of the C.elegans nervous system and also in the developing vulva. Using chemotaxis assays, we show that both ADARs are important for normal behavior. Biochemical, molecular and phenotypic analyses indicate that ADR-1 and ADR-2 have distinct roles in C.elegans, but sometimes act together.


http://embojournal.npgjournals.com/cgi/content/abstract/21/11/2703

Wnt signals regulate differentiation of neural crest cells through the (beta)-catenin associated with a nuclear mediator of the lymphoid-enhancing factor 1 (LEF-1)/T-cell factors (TCFs) family. Here we show the interaction between the basic helix-loop-helix and leucine-zipper region of microphthalmia-associated transcription factor (MITF) and LEF-1. MITF is essential for melanocyte differentiation and its heterozygous mutations cause auditory-pigmentary syndromes. Functional cooperation of MITF with LEF-1 results in synergistic transactivation of the dopachrome tautomerase (DCT) gene promoter, an early melanoblast marker. This activation depends on the separate cis-acting elements, which are also responsible for the induction of the DCT promoter by lithium chloride that mimics Wnt signaling. (beta)-catenin is required for efficient transactivation, but dispensable for the interaction between MITF and LEF-1. The interaction with MITF is unique to LEF-1 and not detectable with TCF-1. LEF-1 also cooperates with the MITF-related proteins, such as TFE3, to transactivate the DCT promoter. This study therefore suggests that the MITF/TFE3 family is a new class of nuclear modulators for LEF-1, which may ensure efficient propagation of Wnt signals in many types of cells.

EMBO Rep.  (1)


http://emboreports.npgjournals.com/cgi/content/abstract/5/10/989

BRCA2 is a breast cancer susceptibility gene implicated in the repair of double-strand breaks by homologous recombination with RAD51. BRCA2 associates with a 70-amino-acid protein, DSS1, but the functional significance of this interaction has remained unclear. Recently, deficiency of a DSS1 orthologue in the fungus Ustilago maydis has been shown to cause a defect in recombinational DNA repair. Here we have investigated the consequences of DSS1 depletion in mammalian cells. We show that like BRCA2, DSS1 is required for DNA damage-induced RAD51
focus formation and for the maintenance of genomic stability, indicating a function conserved from lower eukaryotes to humans. However, DSS1 seems to be not required for BRCA2 or RAD51 stability or for BRCA2 and RAD51 to interact, raising the possibility that DSS1 may be required for the BRCA2-RAD51 complex to become associated with sites of DNA damage.

Endocr. Relat. Cancer (1)


http://erc.endocrinology-journals.org/cgi/content/abstract/12/1/65

We have been investigating gene-expression profiles in estrogen receptor (ER)-negative breast cancers to identify molecules involved in breast carcinogenesis and to select genes or gene products that might be useful as diagnostic markers or targets for new molecular therapies. Here we report evidence that the gene encoding retinoic acid-induced protein 3 (RAI3) is a potential molecular target for treatment of breast cancers. Using quantitative reverse transcription-PCR (RT-PCR), we documented increased expression of RAI3 in 19 of 25 primary breast cancers and in 6 of 11 breast-cancer cell lines examined, by comparison with normal mammary-gland tissue. Treatment of human embryonic kidney (HEK293) cells with siRNA against RAI3 suppressed expression of RAI3 and also suppressed cell growth. Transfection of siRNA into breast-cancer cell lines MCF7 and T47D also suppressed RAI3 mRNA and growth of the cancer cells. Because our data imply that up-regulation of RAI3 function is a frequent feature of breast carcinogenesis, we suggest that selective suppression of signal from RAI3 might hold promise for development of a new strategy for treating breast cancers.

Environment International (1)


http://www.sciencedirect.com/science/article/B6V7X-472BHGG-1/2/494f519500afea5c8aa921ac383b3c9e

The presence of erm genes was investigated among macrolide-resistant Gram-positive bacteria isolated from soil samples collected from four Danish farms that had been treated with animal waste. Soil samples were collected before, a few days after spread and 1 months and 5 months later. In 33% (9/27) of these isolates, an erm gene was detected using PCR. Eight isolates were positive for erm(B) and one isolate was positive for erm(C). No isolates contained erm(A), erm(D) or erm(F). The positive isolates were identified to genus level. Two erm(B) positive isolates were identified as Enterococcus spp., and the erm(C)-positive isolate as a Streptococcus spp., probably indicating survival from animal waste. The remaining six erm(B) positive isolates all belonged to the Bacillus cereus group. The erm(B) gene has not previously been identified in B.
cereus but is frequently found among enterococci. This result could indicate horizontal transfer from bacteria of animal origin to indigenous soil bacteria.

**Environmental Mutagenesis and Related Subjects** (2)


http://www.sciencedirect.com/science/article/B73H5-47RNW7X-4Y/2/b29592180fa2fd00c8e8df584ca00592

We have used the polymerase chain reaction (PCR) to speed the DNA sequence analysis of revertants of Salmonella typhimurium TA98. Briefly, a crude DNA extract from a single colony was prepared and used in an asymmetric PCR to amplify a 328-bp fragment containing the hisD3052 mutation approximately in the center. Following ultrafiltration, the ssDNA was sequenced using an end-labeled probe and dideoxy sequencing. The most frequent mutation among the revertants was a -2 deletion of GC or CG within the sequence CGCGCGCG, which is upstream of the hisD3052 mutation. This deletion occurred in 38% (6/16) of the spontaneous (-S9) revertants and in 94% (15/16) of a set of 1-nitropyrene-induced revertants. Other mutations, mostly deletions but also some complex mutations (i.e., single mutational events involving a combination of insertions, deletions, and substitutions), occurred within quasipalindromic regions of DNA. Possible mutational mechanisms are discussed, and the results with 1-NP are compared to those obtained in other systems.


The lacI gene has been used as a target gene in various mutation assays. We modified single strand conformation polymorphism (SSCP) analysis by introducing restriction digestion to detect mutations in the gene rapidly, and determined the sensitivity of the method. The entire coding sequence and partial promoter region of the lacI gen were amplified by the polymerase chain reaction with ([alpha]-32P)dCTP in a 1247 base pair fragment, digested into eight restriction fragments, and analyzed by SSCP. The sensitivity of the method was assessed using 160 phages with lacI mutations, which were selected by assay of expression of [beta]-galactosidase after their infection into E. coli. Of the 160 mutants, 146 (91.3%) showed shifted bands in the first condition of SSCP analysis (without glycerol, 20[deg]C). The remaining 14 mutants were analyzed in a second condition (with 5% glycerol, 20[deg]C), and eight of them showed shifted bands (cumulatively 96.3% of the 160 mutants). The remaining six mutants were analyzed in a third condition (with 5% glycerol, 10[deg]C), and all of them showed shifted bands (cumulatively 100%). Sequencing of the restriction fragments with mobility shifts in the 160 mutants revealed 108 kinds of mutations, 100 (92.6%) being detected in the first condition, seven (cumulatively
99.1%) in the second condition, and one (cumulatively 100%) in the third condition. This method greatly reduced the time to identify lacI mutations, and allowed the detection of multiple mutations in one lacI mutant. The results also show that in general PCR-SSCP analysis is very sensitive when test fragments are shorter than about 250 base pairs and electrophoresis is performed under at least two conditions.

Environmental Toxicology and Pharmacology  (1)


http://www.sciencedirect.com/science/article/B6T6D-41Y876R-1/2/879c04aec3fc41ddf598e5a2c382b2f1

Diesel exhaust particles (DEP) are assumed to be a causal substance for pulmonary inflammation. As peroxynitrite is recently implicated in inflammation and cytotoxicity, the hypothesis was tested that instillation of DEP induces formation of peroxynitrite in cells migrated in lung. Rats were intratracheally instilled with DEP suspension (2 mg/0.5 ml/kg) and killed 24 h later. Alveolar cells were collected by broncho-alveolar lavage. Population of alveolar cells increased more than twice by DEP exposure, mainly due to a large increase of neutrophils. Peroxynitrite formation (NG-nitro--arginine methylester and superoxide dismutase inhibitable chemiluminescence) was detected in alveolar cells from treated rats, and 12-O-tetradecanoylphorbol 13-acetate-stimulation enhanced it. In addition, DEP induced expression of inducible NO synthase mRNA in these cells. But peroxynitrite was not detectable in cells from control. These results indicate that DEP exposure results in peroxynitrite formation in migrated cells, which leads to pulmonary inflammation.

Enzyme and Microbial Technology  (2)


http://www.sciencedirect.com/science/article/B6TG1-47MJ556-1/2/4019c86313b6741d32f27162e9d9c1ae

Five isolates able to utilize dibenzothiophene (DBT) as a sole sulfur source and to convert it to 2-hydroxybiphenyl (HBP) with high rates were selected to investigate their potentialities as biocatalysts of a diesel oil biodesulfurization process. Conventional and chemotaxonomic analyses and 16S ribosomal DNA (rDNA) sequencing showed that these strains belonged to the Rhodococcus/Gordonia cluster. The desulfurizing activities of resting cells were compared under various conditions to evaluate their stability in both aqueous and organic media, their sensitivity to the presence of hexadecane and their sulfur substrate selectivity. In spite of their taxonomic
similarity, the five strains exhibited different properties. This diversity was not confirmed by the analysis of the desulfurizing genes by amplification and sequencing of large fragments of dszA, dszB, dszC, and dszD genes which revealed that four of the five selected strains had a dsz genotype identical to those of the reference strain, Rhodococcus erythropolis IGTS8.


On the past, Taq polymerase was reversibly inactivated by modification with a dicarboxylic acid anhydride in aqueous media, to enable 'hot start PCR'. However, there are various constraints in using such a method including temperature and concentration. Here we describe an alternative method whereby Taq polymerase may be reversibly inactivated following incubation with an excess of citraconic anhydride at elevated temperatures, in an anhydrous non-protic organic solvent - tert-butyl methyl ether - by first drying the enzyme with a salt or carbohydrate excipient to form an amorphous powder. Reactivation of the enzyme is due to the instability of the chemical modification at low pH following a short incubation in a suitable buffer.

Epilepsy Research (3)


http://www.sciencedirect.com/science/article/B6T34-48CGF7H-1/2/0c3c698833f1497d0b0cd5c1747d2de4

Febrile convulsions (FCs) represent the majority of childhood seizures, and patients have a genetic predisposition to their development. The genetic susceptibility to FCs seems to involve multiple genes in most instances. Recent studies provided evidence that mutations in SCN1A represent the most frequent cause of generalized epilepsy with febrile seizures plus an autosomal-dominant epilepsy syndrome. SCN1A mutations alter channel inactivation, resulting in persistent inward sodium current. It is not known if polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FCs. By performing an association study, we used single nucleotide polymorphisms to investigate the distribution of genotypes of SCN1A in patients with FCs. A total of 104 Taiwanese children with FCs and 83 normal control subjects were included in the study. Polymerase chain reaction was used to identify the A/G polymorphism of the SCN1A gene. The results showed that genotypes and allelic frequencies for the SCN1A gene polymorphisms in both groups were not significantly different. These data suggest that the SCN1A gene might not be one of the susceptibility factors for FCs. Pure FCs and febrile convulsions associated with idiopathic generalized epilepsy may not share a common genetic etiology.

Various studies have shown that brain-derived neurotrophic factor (BDNF) increased neuronal excitability. We tested that BDNF might be involved in the etiology of febrile seizures (FSs). A total of 186 Taiwanese children were divided into two groups: (1) FSs (n = 104); (2) normal control subjects (n = 83). A single base pair polymorphism SNP6265 (Val66Met) at position 196 was analyzed. Our findings suggest that the BDNF polymorphisms were not candidate genetic markers.


Disruption of the function of the mouse jerky gene by transgene insertion causes generalized recurrent seizures reminiscent of human idiopathic generalized epilepsy (IGE). A human homologue, JRK/JH8, has been cloned, which maps to 8q24, a chromosomal region associated with several forms of IGE. JRK/JH8 is, therefore, a candidate locus for at least some forms of IGE. We report corrected cDNA sequences and extended open reading frames for the mouse jerky and human JRK/JH8 genes, which add 48 amino acids to the N-terminus of the Jerky protein and which extends the region of homology with the N-terminal DNA-binding domain of the centromere-binding protein, CENP-B. Systematic sequencing of the coding region of the extended JRK/JH8 gene identified single nucleotide polymorphisms that define three haplotypes, which were used for association studies in patients with idiopathic generalized epilepsy. We report one subject with childhood absence epilepsy (CAE) that evolved to juvenile myoclonic epilepsy (JME) that has a unique de novo mutation that results in a non-conservative amino acid change at a potential protein glycosylation site. Familial analysis supports a causal role for this mutation in the disease.

Eukaryot. Cell (2)


We describe a novel gene family that forms clusters in subtelomeric regions of Trypanosoma brucei chromosomes and partially accounts for the observed clustering of retrotransposons. The ingi and ribosomal inserted mobile element (RIME) non-LTR retrotransposons share 250 bp at both extremities and are the most abundant putatively mobile elements, with about 500 copies per haploid genome. From cDNA clones and subsequently in the T. brucei genomic DNA databases, we identified 52 homologous gene and pseudogene sequences, 16 of which contain a RIME and/or ingi retrotransposon inserted at exactly the same relative position. Here these genes
are called the RHS family, for retrotransposon hot spot. Comparison of the protein sequences encoded by RHS genes (21 copies) and pseudogenes (24 copies) revealed a conserved central region containing an ATP/GTP-binding motif and the RIME/ingi insertion site. The RHS proteins share between 13 and 96% identity, and six subfamilies, RHS1 to RHS6, can be defined on the basis of their divergent C-terminal domains. Immunofluorescence and Western blot analyses using RHS subfamily-specific immune sera show that RHS proteins are constitutively expressed and occur mainly in the nucleus. Analysis of Genome Survey Sequence databases indicated that the Trypanosoma brucei diploid genome contains about 280 RHS (pseudo)genes. Among the 52 identified RHS (pseudo)genes, 48 copies are in three RHS clusters located in subtelomeric regions of chromosomes Ia and II and adjacent to the active bloodstream form expression site in T. brucei strain TREU927/4 GUTat10.1. RHS genes comprise the remaining sequence of the size-polymorphic "repetitive region" described for T. brucei chromosome I, and a homologous gene family is present in the Trypanosoma cruzi genome.


http://ec.asm.org/cgi/content/abstract/1/6/895

The dimorphic fungi Blastomyces dermatitidis and Histoplasma capsulatum cause systemic mycoses in humans and other animals. Forward genetic approaches to generating and screening mutants for biologically important phenotypes have been underutilized for these pathogens. The plant-transforming bacterium Agrobacterium tumefaciens was tested to determine whether it could transform these fungi and if the fate of transforming DNA was suited for use as an insertional mutagen. Yeast cells from both fungi and germinating conidia from B. dermatitidis were transformed via A. tumefaciens by using hygromycin resistance for selection. Transformation frequencies up to 1 per 100 yeast cells were obtained at high effector-to-target ratios of 3,000:1. B. dermatitidis and H. capsulatum ura5 lines were complemented with transfer DNA vectors expressing URA5 at efficiencies 5 to 10 times greater than those obtained using hygromycin selection. Southern blot analyses indicated that in 80% of transformants the transferred DNA was integrated into chromosomal DNA at single, unique sites in the genome. Progeny of B. dermatitidis transformants unexpectedly showed that a single round of colony growth under hygromycin selection or visible selection of transformants by lacZ expression generated homokaryotic progeny from multinucleate yeast. Theoretical analysis of random organelle sorting suggests that the majority of B. dermatitidis cells would be homokaryons after the ca. 20 generations necessary for colony formation. Taken together, the results demonstrate that A. tumefaciens efficiently transfers DNA into B. dermatitidis and H. capsulatum and has the properties necessary for use as an insertional mutagen in these fungi.

Eur. Heart J. (2)


http://eurheartj.oupjournals.org/cgi/content/abstract/26/6/584
Aims To assess, in families with premature coronary artery disease (CAD), the possible association, with linkage, of the X-linked AT2 receptor (-1332 G/A) gene polymorphism and premature CAD. Methods and results We investigated 509 families with a history of premature CAD that consisted of one sibling affected with premature CAD and two unaffected siblings. Genotyping of subjects was performed using a restriction enzyme digestion of an initial 310 bp polymerase chain reaction fragment that included the AT2 (-1332 G/A) locus. The mean age of the 611 individuals affected by premature CAD at the time of event was 49.5±8.1 years. Conditional logistic regression analysis confirmed a significant predictive value of premature CAD for the covariates of hypertension, diabetes, dyslipidaemia, history of smoking, and male gender. The genetic data were analysed for these families using the X-linked sibling transmission/deletion test (XS-TDT) statistics program. In hemizygous men we observed evidence for association in the presence of linkage, for the AT2 (-1332 G/A) locus and premature CAD (P-exact value=0.024) and also a trend towards association, in the presence of linkage, for this polymorphism and hypertension (P-exact value=0.08). Conclusions We have observed evidence of association between the presence of linkage for the X-linked AT2 (-1332 G/A) polymorphism and premature CAD in hemizygous males.


http://eurheartj.oupjournals.org/cgi/content/abstract/25/5/377

Aims We tested the hypothesis that cardiac angiotensin II (Ang II) receptor gene transcription may predict the development of transplant coronary artery disease (TCAD) following heart transplantation. Methods and results We examined the gene transcripts of Ang II type 1 (AT1R) and type 2 receptors (AT2R) in endomyocardial biopsy specimens from 50 heart transplant recipients. The progression of TCAD was measured as change in maximal intimal thickness (CMIT) and change in plaque volume (CPV) by intravascular ultrasound (IVUS) examinations from baseline to one year after transplantation. The development of transplant vasculopathy was defined as a CMIT of ≥0.3 mm over one year. The level of AT1R mRNA was associated with that of AT2R in transplanted hearts (regression coefficient=1.77, 95% CI 0.85-2.89, [IMG]f1.gif" BORDER="0">). AT1R and AT2R gene transcripts were univariate predictors of CMIT (AT1R: regression coefficient 0.10, 95% CI 0.06-0.14, [IMG]f1.gif" BORDER="0">; AT2R: regression coefficient 0.28, 95% CI 0.17-0.40, [IMG]f1.gif" BORDER="0">) or CPV (AT1R: regression coefficient 0.41, 95% CI 0.17-0.65, [IMG]f1.gif" BORDER="0">; AT2R: regression coefficient 1.25, 95% CI 0.49-2.01, [IMG]f2.gif" BORDER="0">). By one year, 21 (46%) transplant recipients showed evidence of transplant vasculopathy and the rest did not. The vasculopathic group demonstrated a higher level of expression of cardiac AT1R than the non-vasculopathic group (3.7±2.9 vs 1.6±1.7 folds; [IMG]f3.gif" BORDER="0">). The level of AT1R mRNA in transplanted heart was identified as a discriminator that predicted the development of transplant vasculopathy with a sensitivity of 75% and specificity of 83%. Conclusions Cardiac Ang II receptor gene transcripts are associated with the progression of TCAD following heart transplantation. Only AT1R gene transcripts predicted the development of transplant vasculopathy in this preliminary study. These findings potentially support a role of Ang II receptors in the progression of TCAD following cardiac transplantation.

Eur. J. Biochem. (15)

http://content.febsjournal.org/cgi/content/abstract/269/4/1293

The {alpha}s1 -casein (\(\alpha_s1\)-Cas) locus in the goat is characterized by a polymorphism, the main feature of which is to be qualitative as well as quantitative. A systematic analysis performed in an autochthon southern Italy breed identified a new rare allele (M), which was characterized at both the protein and genomic level. The M protein displays the slowest electrophoretic mobility of the {alpha}s1 -Cas variants described so far. MS and automated Edman degradation experiments showed that this behavior was due to the loss of two phosphate residues in the multiple phosphorylation site (64SP -SP -SP -SP -SP -E-70E) consecutively to a Ser[-\gt]Leu substitution at position 66 of the peptide chain (64S-SP -L-SP -SP -E-70E). This was confirmed by sequencing a genomic DNA fragment encompassing exon 9 where the 8th codon (TCG) was shown to be mutated to TTG. Sequencing of amplified genomic DNA segments spanning the 5' and 3' flanking regions of each exon allowed us to identify 23 single nucleotide polymorphisms and two insertion/deletion events in the coding as well as the noncoding regions. A comparison of specific haplotypes defined for each of the\(\alpha_s1\)-CasF, A and M alleles indicates that the M allele probably arises from interallelic recombination between alleles A and B 2, followed by a C[-\gt]T transition at nucleotide 23 of the ninth exon. The region encompassing the recombination break point was putatively located between nucleotide 86 upstream and nucleotide 40 downstream of exon 8. Interallelic recombination therefore appears to be a possible means of generating allelic diversity at the\(\alpha_s1\)-Cas locus, at least in the goat. The previously proposed molecular phylogeny must now be revised, possibly starting from two ancestral allelic lineages.


http://content.febsjournal.org/cgi/content/abstract/269/18/4566

Thyrotropin-releasing hormone receptor (TRHR) has already been cloned in mammals where thyrotropin-releasing hormone (TRH) is known to act as a powerful stimulator of thyroid-stimulating hormone (TSH) secretion. The TRH receptor of amphibians has not yet been characterized, although TRH is specifically important in the adaptation of skin color to environmental changes via the secretion of \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH). Using a degenerate PCR strategy, we report on the isolation of three distinct cDNA species encoding TRHR from the brain of Xenopus laevis. We have designated these as xTRHR1, xTRHR2 and xTRHR3. Analysis of the predicted amino acid sequences revealed that the three Xenopus TRHRs are only 54-62% identical and contain all the highly conserved residues constituting the TRH binding pocket. Amino acid sequences and phylogenetic analysis revealed that xTRHR1 is a member of TRHR subfamily 1 and xTRHR2 belongs to subfamily 2, while xTRHR3 is a new TRHR subtype awaiting discovery in other animal species. The three Xenopus TRHRs have distinct patterns of expression. xTRHR3 was abundant in the brain and much scarcer in the peripheral tissues, whereas xTRHR1 was found mainly in the stomach and xTRHR2 in the heart. The Xenopus TRHR subtype 1 was found specifically in the intestine, lung and urinary bladder. These observations suggest that the three xTRHRs each have specific functions that remain to be elucidated. Expression in Xenopus oocytes and HEK-293 cells indicates that the three Xenopus TRHRs are fully functional and are coupled to the inositol phosphate/calcium pathway. Interestingly, activation of xTRHR3 required larger concentrations of TRH compared with the other two receptors, suggesting marked differences in receptor binding, coupling or regulation.
Several clinical trials have revealed that individuals who were given (beta)-carotene and vitamin A did not have a reduced risk of cancer compared to those given placebo; rather, vitamin A could actually have caused an adverse effect in the lungs of smokers [Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S. & Hammar, S. N. Engl. J. Med (1996) 334, 1150-1155; Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., LaMotte, F., Gaziano, J.M., Ridker, P.M., Willet, W. & Peto, R. (1996) N. Engl. J. Med. 334, 1145-1149]. Using differential display techniques, an initial survey using rats showed that liver RNA expression of c-H-Ras was decreased and p53 increased in rats with chronic vitamin A deficiency. These findings prompted us to evaluate the expression of c-Jun, p53 and p21 WAF1/CIF1 (by RT-PCR) in liver and lung of rats. This study showed that c-Jun levels were lower and that p53 and p21 WAF1/CIF1 levels were higher in chronic vitamin A deficiency. Vitamin A supplementation increased expression of c-Jun, while decreasing the expression of p53 and p21 WAF1/CIF1. Western-blot analysis demonstrated that c-Jun and p53 showed a similar pattern to that found in the RT-PCR analyses. Binding of retinoic acid receptors (RAR) to the c-Jun promoter was decreased in chronic vitamin A deficiency when compared to control hepatocytes, but contrasting results were found with acute vitamin A supplemented cells. DNA fragmentation and cytochrome c release from mitochondria were analyzed and no changes were found. In lung, an increase in the expression of c-Jun produced a significant increase in cyclin D1 expression. These results may explain, at least in part, the conflicting results found in patients supplemented with vitamin A and illustrate that the changes are not restricted to lung. Furthermore, these results suggest that pharmacological vitamin A supplementation may increase the risk of adverse effects including the risk of oncogenesis.


Phospholipase D (PLD) plays a major role in the activation of the neutrophil respiratory burst. However, the repertoire of PLD isoforms present in these primary cells, the precise mechanism of activation, and the impact of cell priming on PLD activity and localization remain poorly defined. RT-PCR analysis showed that both PLD1 and PLD2 isoforms are expressed in human neutrophils, with PLD1 expressed at a higher level. Endogenous PLD1 was detected by immunoprecipitation and Western blotting, and was predominantly membrane-associated under control and primed/stimulated conditions. Immunofluorescence showed that PLD had a punctate distribution throughout the cell, which was not altered after stimulation by soluble agonists. In contrast, PLD localized to the phagolysosome membrane after ingestion of nonopsonized zymosan particles. We also demonstrate that tumour necrosis factor {alpha} greatly potentiates agonist-stimulated PLD activation, myeloperoxidase release, and superoxide anion generation, and that PLD activation occurs via a phosphatidylinositol 3-kinase-sensitive and brefeldin-sensitive ADP-ribosylation factor GTPase-regulated mechanism. Moreover, propranolol, which causes an increase in PLD-derived phosphatidic acid accumulation, caused a selective increase in agonist-stimulated myeloperoxidase release. Our results indicate that priming is a critical regulator of PLD activation, that the PLD-generated lipid products exert divergent effects on neutrophil functional responses, that PLD1 is the major PLD isoform present in human
neutrophils, and that PLD1 actively translocates to the phagosomal wall after particle ingestion.


http://content.febsjournal.org/cgi/content/abstract/271/1/108

Volkensin, a type 2 ribosome-inactivating protein from the roots of Adenia volkensii Harms (kilyambiti plant) was characterized both at the protein and nucleotide level by direct amino acid sequencing and cloning of the gene encoding the protein. Gene sequence analysis revealed that volkensin is encoded by a 1569-bp ORF (523 amino acid residues) without introns, with an internal linker sequence of 45 bp. Differences in residues present at several sequence positions (reproduced after repeated protein sequence analyses), with respect to the gene sequence, suggest several isoforms for the volkensin A-chain. Based on the crystallographic coordinates of ricin, which shares a high sequence identity with volkensin, a molecular model of volkensin was obtained. The 3D model suggests that the amino acid residues of the active site of the ricin A-chain are conserved at identical spatial positions, including Ser203, a novel amino acid residue found to be conserved in all known ribosome-inactivating proteins. The sugar binding site 1 of the ricin B-chain is also conserved in the volkensin B-chain, whilst in binding site 2, His246 replaces Tyr248. Native volkensin contains two free cysteinyl residues out of 14 derived from the gene sequence, thus suggesting a further disulphide bridge in the B chain, in addition to the intrachain disulphide bond pattern common to other type 2 ribosome-inactivating proteins.


http://content.febsjournal.org/cgi/content/abstract/269/24/6162

The marine snail Conus is the sole invertebrate wherein both the vitamin K-dependent carboxylase and its product, (gamma)-carboxyglutamic acid, have been identified. To examine its biosynthesis of (gamma)-carboxyglutamic acid, we studied the carboxylase from Conus venom ducts. The carboxylase cDNA from Conus textile has an ORF that encodes a 811-amino-acid protein which exhibits sequence similarity to the vertebrate carboxylases, with 41% identity and (approx) 60% sequence similarity to the bovine carboxylase. Expression of this cDNA in COS cells or insect cells yielded vitamin K-dependent carboxylase activity and vitamin K-dependent epoxidase activity. The recombinant carboxylase has a molecular mass of (approx) 130 kDa. The recombinant Conus carboxylase carboxylated Phe-Leu-Glu-Glu-Leu and the 28-residue peptides based on residues -18 to +10 of human prothrombin and proFactor IX with K m values of 420 (micro)m, 1.7 (micro)m and 6 (micro)m, respectively; the K m for vitamin K is 52 (micro)m. The K m values for peptides based on the sequence of the conotoxin (epsilon)-TxlX and two precursor analogs containing 12 or 29 amino acids of the propeptide region are 565 (micro)m, 75 (micro)m and 74 (micro)m, respectively. The recombinant Conus carboxylase, in the absence of endogenous substrates, is stimulated up to fivefold by vertebrate propeptides but not by Conus propeptides. These results suggest two propeptide-binding sites in the carboxylase, one that binds the Conus and vertebrate propeptides and is required for substrate binding, and the other that binds only the vertebrate propeptide and is required for enzyme stimulation. The marked functional and structural similarities between the Conus carboxylase and vertebrate vitamin K-dependent (gamma)-carboxylases argue for conservation of a vitamin K-dependent carboxylase across animal species and the importance of (gamma)-carboxyglutamic acid synthesis in diverse biological systems.

http://content.febsjournal.org/cgi/content/abstract/270/1/163

The ST2 gene, which is specifically induced by growth stimulation in fibroblasts, encodes interleukin-1 receptor-related proteins and is widely expressed in hematopoietic, helper T, and various cancer cells. However, the physiological as well as pathological functions of the ST2 gene products are not yet fully understood. In this study, we analyzed the expression of the ST2 gene in human glioma cell lines and human brain tumor samples with real-time polymerase chain reaction method, the results of which revealed that the expression level of the ST2 gene in glioma cell lines and glioblastoma samples is significantly lower than that in a fibroblastic cell line, TM12, and benign brain tumors, suggesting the reverse relationship between malignancy and ST2 expression. As we could not detect the soluble ST2 protein in the culture fluid of the T98G glioblastic cell line by ELISA, we established stable transformants of T98G that continuously produce and secrete the ST2 protein, in order to study the effect of the ST2 protein on malignancy. Although we could not detect a remarkable difference in proliferation between transformants and control cells in conventional tissue culture dishes, the efficiency of colony formation in soft agar was significantly decreased in the case of cells that continuously produce the ST2 protein. Furthermore, inhibition of colony formation in soft agar was observed in wild-type T98G cells when purified soluble ST2 protein was added to the culture, in a dose-dependent manner. Taken together, the results suggest that the expression of ST2 suppressed the anchorage-independent growth and malignancy.


http://content.febsjournal.org/cgi/content/abstract/269/21/5137

The structurally homologous mononuclear iron and manganese superoxide dismutases (FeSOD and MnSOD, respectively) contain a highly conserved glutamine residue in the active site which projects toward the active-site metal centre and participates in an extensive hydrogen bonding network. The position of this residue is different for each SOD isoenzyme (Q69 in FeSOD and Q146 in MnSOD of Escherichia coli). Although site-directed mutant enzymes lacking this glutamine residue (FeSOD[Q69G] and MnSOD[Q146A]) demonstrated a higher degree of selectivity for their respective metal, they showed little or no activity compared with wild types. FeSOD double mutants (FeSOD[Q69G/A141Q]), which mimic the glutamine position in MnSOD, elicited 25% the activity of wild-type FeSOD while the activity of the corresponding MnSOD double mutant (MnSOD[G77Q/Q146A]) increased to 150% (relative to wild-type MnSOD). Both double mutants showed reduced selectivity toward their metal. Differences exhibited in the thermostability of SOD activity was most obvious in the mutants that contained two glutamine residues (FeSOD[A141Q] and MnSOD[G77Q]), where the MnSOD mutant was thermostable and the FeSOD mutant was thermostable. Significantly, the MnSOD double mutant exhibited a thermal-inactivation profile similar to that of wild-type FeSOD while that of the FeSOD double mutant was similar to wild-type MnSOD. We conclude therefore that the position of this glutamine residue contributes to metal selectivity and is responsible for some of the different physicochemical properties of these SODs, and in particular their characteristic thermostability.

http://content.febsjournal.org/cgi/content/abstract/271/13/2584

A novel hypoxically regulated intercellular junction protein (claudin-like protein of 24 kDa, CLP24) has been identified that shows homology to the myelin protein 22/epithelial membrane protein 1/claudin family of cell junction proteins, which are involved in the modulation of paracellular permeability. The CLP24 protein contains four predicted transmembrane domains and a C-terminal protein-protein interaction domain. These domains are characteristic of the four transmembrane spanning (tetraspan) family of proteins, which includes myelin protein 22, and are involved in cell adhesion at tight, gap and adherens junctions. Expression profiling analyses show that CLP24 is highly expressed in lung, heart, kidney and placental tissues. Cellular studies confirm that the CLP24 protein localizes to cell-cell junctions and co-localizes with the (beta)-catenin adherens junction-associated protein but not with tight junctions. Over-expression of CLP24 results in decreased adhesion between cells, and functional paracellular flux studies confirm that over-expression of the CLP24 protein modulates the junctional barrier function. These data therefore suggest that CLP24 is a novel, hypoxically regulated tetraspan adherens junction protein that modulates cell adhesion, paracellular permeability and angiogenesis.


http://content.febsjournal.org/cgi/content/abstract/270/2/230

Human (beta)2-glycoprotein I ((beta)2GPI), also known as apolipoprotein H, has been implicated in haemostasis and the production of anti-phospholipid antibodies. There is a wide range of interindividual variation in (beta)2GPI plasma levels that is thought to be under genetic control, but its molecular basis remains unknown. To understand the genetic basis of (beta)2GPI variation, we analyzed the 5' flanking region of the (beta)2GPI gene for mutation detection by DHPLC and identified a point mutation at the transcriptional initiation site (-1C[gt]A) with a carrier frequency of 12.1%. The mutation was associated with significantly lower (beta)2GPI plasma levels (P < 0.0001) and low occurrence of anti-phospholipid antibodies in lupus patients (4.8% antibody-positive group vs. 16.6% in the antibody-negative group; P = 0.019). Northern blot analysis confirmed that the -1C[gt]A mutation was associated with lower mRNA levels and it reduced the reporter (luciferase) gene expression by twofold. Electrophoretic gel mobility shift assay (EMSA) revealed that the -1C[gt]A mutation disrupts the binding for crude hepatic nuclear extracts and purified TFIID. These results suggest that the substitution of C with A at the (beta)2GPI transcriptional initiation site is a causative mutation that affects its gene expression at the transcriptional level and ultimately (beta)2GPI plasma levels and the occurrence of anti-phospholipid antibodies.


http://content.febsjournal.org/cgi/content/abstract/270/2/213

We report the cloning, expression, pharmacological characterization and tissue distribution of a melanocortin (MC) receptor gene in a shark, the spiny dogfish (Squalus acanthias) (Sac).
Phylogenetic analysis showed that this receptor is an ortholog of the MC4 subtype, sharing 71% overall amino acid identity with the human (Hsa) MC4 receptor. When expressed and characterized by radioligand binding assay for the natural MSH (melanocyte-stimulating hormone) peptides {alpha}-, {beta}-, and {gamma}-MSH, the SacMC4 receptor showed pharmacological properties very similar to the HsaMC4 receptor. Stimulation of SacMC4 receptor transfected cells with {alpha}-MSH caused a dose-dependent increase in intracellular cAMP levels. The SacMC4 receptor has Ala in position 59 where all other cloned MC receptors have Glu. We confirmed that this was not due to individual polymorphism and subsequently mutated the residue back to Glu but the mutation did not affect the pharmacological properties of the receptor. SacMC4 receptor mRNA was detected by RT-PCR in the optic tectum, hypothalamus, brain stem, telencephalon and olfactory bulb but not in cerebellum or in peripheral tissues. This study describes the first characterization of an MC receptor in a cartilaginous fish, the most distant MC receptor gene cloned to date. Conservation of gene structure, pharmacological properties and tissue distribution suggests that this receptor may have similar roles in sharks as in mammals and that these were established more than 450 million years ago.


http://content.febsjournal.org/cgi/content/abstract/269/24/6052

Bothrops snake venoms are known to induce local tissue damage such as hemorrhage and myonecrosis. The opossum Didelphis marsupialis is resistant to these snake venoms and has natural venom inhibitors in its plasma. The aim of this work was to clone and study the chemical, physicochemical and biological properties of DM64, an ant.myotoxmic protein from opossum serum. DM64 is an acidic protein showing 15% glycosylation and with a molecular mass of 63 659 Da when analysed by MALDI-TOF MS. It was cloned and the amino acid sequence was found to be homologous to Dm43, a metalloproteinase inhibitor from D. marsupialis serum, and to human {alpha}1 B-glycoprotein, indicating the presence of five immunoglobulin-like domains. DM64 neutralized both the in vivo myotoxicity and the in vitro cytotoxicity of myotoxins I (mt-I/Asp49) and II (mt-II/Lys49) from Bothrops asper venom. The inhibitor formed noncovalent complexes with both toxins, but did not inhibit the PLA2 activity of mt-I. Accordingly, DM64 did not neutralize the anticoagulant effect of mt-I nor its intracerebroventricular lethality, effects that depend on its enzymatic activity, and which demonstrate the dissociation between the catalytic and toxic activities of this Asp49 myotoxic PLA2. Furthermore, despite its similarity with metalloproteinase inhibitors, DM64 presented no antihemorrhagic activity against Bothrops jararaca or Bothrops asper crude venoms, and did not inhibit the fibrinogenolytic activity of jararhagin or bothrolysin. This is the first report of a myotoxin inhibitor with an immunoglobulin-like structure isolated and characterized from animal blood.


http://content.febsjournal.org/cgi/content/abstract/269/18/4387

Retinyl esters are a major endogenous storage source of vitamin A in vertebrates and their hydrolysis to retinol is a key step in the regulation of the supply of retinoids to all tissues. Some members of nonspecific carboxylesterase family (EC 3.1.1.1) have been shown to hydrolyze retinyl esters. However, the number of different isoenzymes that are expressed in the liver and their retinyl palmitate hydrolase activity is not known. Six different carboxylesterases were identified and purified from rat liver microsomal extracts. Each isoenzyme was identified by mass
spectrometry of its tryptic peptides. In addition to previously characterized rat liver carboxylesterases ES10, ES4, ES3, the protein products for two cloned genes, AB010635 and D50580 (GenBank accession numbers), were also identified. The sixth isoenzyme was a novel carboxylesterase and its complete cDNA was cloned and sequenced (AY034877). Three isoenzymes, ES10, ES4 and ES3, account for more than 95% of rat liver microsomal carboxylesterase activity. They obey Michaelis-Menten kinetics for hydrolysis of retinyl palmitate with Km values of about 1 {micro}m and specific activities between 3 and 8 nmol{middle dot}min-1{middle dot}mg-1 protein. D50580 and AY034877 also hydrolyzed retinyl palmitate. Gene-specific oligonucleotide probing of multiple-tissue Northern blot indicates differential expression in various tissues. Multiple genes are highly expressed in small intestine, important tissues for retinoid metabolism. The level of expression of any one of the six different carboxylesterase isoenzymes will regulate the metabolism of retinyl palmitate in specific rat cells and tissues.


http://content.febsjournal.org/cgi/content/abstract/269/22/5527

A novel cosmid (pABC6.5) whose DNA insert from Streptomyces capreolus, the A201A antibiotic producer, overlaps the inserts of the previously reported pCAR11 and pCAR13 cosmids, has been isolated. These two latter cosmids were known to contain the aminonucleoside antibiotic A201A resistance determinants ard2 and ard1, respectively. Together, these three cosmids have permitted the identification of a DNA stretch of 19 kb between ard1 and ard2, which should comprise a large region of a putative A201A biosynthetic (ata) gene cluster. The sequence of the 7 kb upstream of ard1 towards ard2 reveals seven consecutive open reading frames: ataP3, ataP5, ataP4, ataP10, ataP7, ata12 and ataPKS1. Except for the last two, their deduced products present high similarities to an identical number of counterparts from the pur cluster of Streptomyces albogulisper that were either known or proposed to be implicated in the biosynthesis of the N6,N6-dimethyl-3'-amino-3'-deoxyadenosine moiety of puromycin. Because A201A contains this chemical moiety, these ataP genes are most likely implicated in its biosynthesis. Accordingly, the ataP4, ataP5 and ataP10 genes complemented specific puromycin nonproducing (Delta)pur4, (Delta)pur5 and (Delta)pur10 mutants of S. albogulisper, respectively. Amino acid sequence comparisons suggest that ata12 and ataPKS1 could be implicated in the biosynthesis of the d-rhamnose and {alpha}-p-coumaric acid moieties of A201A. Further sequencing of 2 kb of DNA downstream of ard1 has disclosed a region which might contain one end of the ata cluster.


http://content.febsjournal.org/cgi/content/abstract/269/21/5119

Campylobacter jejuni infections are one of the leading causes of human gastroenteritis and are suspected of being a precursor to Guillain-Barre and Miller-Fisher syndromes. Recently, the complete genome sequence of C. jejuni NCTC 11168 was described. In this study, the molecular structure of the lipooligosaccharide and capsular polysaccharide of C. jejuni NCTC 11168 was investigated. The lipooligosaccharide was shown to exhibit carbohydrate structures analogous to the GM1a and GM2 carbohydrate epitopes of human gangliosides (shown below): [IMG]medium/ebj3201.fu1.gif" The high Mr capsule polysaccharide was composed of {beta}-d-Ribp, {beta}-d-GalfNAc, {alpha}-d-GlcpA6(NGro), a uronic acid amidated with 2-amino-2-deoxygycerol
at C-6, and 6-O-methyl-d-glycero-{alpha}-l-gluco-heptopyranose as a side-branch (shown below):

The structural information presented here will aid in the identification and characterization of specific enzymes that are involved in the biosynthesis of these structures and may lead to the discovery of potential therapeutic targets. In addition, the correlation of carbohydrate structure with gene complement will aid in the elucidation of the role of these surface carbohydrates in C. jejuni pathogenesis.

Eur. J. Endocrinol.  (1)


http://www.eje-online.org/cgi/content/abstract/152/4/545

Background: Cyclooxygenase-2 (COX-2) seems to play a role in the development and carcinogenesis of papillary thyroid carcinoma. Its incidence of expression and potential application as a tumor marker remain to be elucidated. Materials and methods: Immunohistochemical staining for COX-2 expression was performed for 30 papillary thyroid carcinoma (PTC) and 40 benign thyroid specimens. COX-2 mRNA expression was analyzed using a reverse transcriptase-polymerase chain reaction (RT-PCR) for paired fresh frozen tissues removed from surgically resected PTC specimens. Results: COX-2 expression was detected by immunohistochemistry in 27 of 30 (90%) PTC but was absent in 40 benign thyroid specimens, including 27 nodular hyperplasia, 7 follicular adenoma and 6 lymphocytic thyroiditis. Two of the three COX-2 negative carcinomas were follicular variant of PTC. RT-PCR analysis confirmed COX-2 mRNA over-expression in 14 of 20 (70%) paired specimens of PTC. Real-time quantitative RT-PCR showed that the level of COX-2 mRNA expression was significantly higher in PTC than in both the adjacent non-cancerous tissues and the benign thyroid specimens. Conclusion: COX-2 is frequently expressed in PTC but not in benign thyroid specimens. COX-2 expression may serve as a useful molecular marker for PTC in cases of diagnostic difficulty.

Eur. Respir. J.  (2)


http://erj.ersjournals.com/cgi/content/abstract/24/1/30

Mutations in the surfactant protein C gene (SFTPC) were recently reported in patients with interstitial lung disease. In a 13-month-old infant with severe respiratory insufficiency, a lung biopsy elicited combined histological patterns of nonspecific interstitial pneumonia and pulmonary alveolar proteinosis. Immunohistochemical and biochemical analyses showed an intra-alveolar accumulation of surfactant protein (SP)-A, precursors of SP-B, mature SP-B, aberrantly processed proSP-C, as well as mono- and dimeric SP-C. Sequencing of genomic DNA detected
a de novo heterozygous missense mutation of the SFTPC gene (g.1286T>C) resulting in a substitution of threonine for isoleucine (I73T) in the C-terminal propeptide. At the ultrastructural level, abnormal transport vesicles were detected in type-II pneumocytes. Fusion proteins, consisting of enhanced green fluorescent protein and wild-type or mutant proSP-C, were used to evaluate protein trafficking in vitro. In contrast to wild-type proSP-C, mutant proSP-C was routed to early endosomes when transfected into A549 epithelial cells. In contrast to previously reported mutations, the I73T represents a new class of surfactant protein C gene mutations, which is marked by a distinct trafficking, processing, palmitoylation, and secretion of the mutant and wild-type surfactant protein C. This report heralds the emerging diversity of phenotypes associated with the expression of mutant surfactant C proteins.


http://erj.ersjournals.com/cgi/content/abstract/22/2/317

Links between immune responses to respiratory syncytial virus (RSV), age and atopic sensitisation are poorly understood. This study investigated the induction of target organ type-1, type-2 and pro-inflammatory cytokine responses to RSV and/or phytohaemagglutinin (PHA) in tonsillar mononuclear cells from children, in relation to age and atopic status. In comparison with the control medium, RSV induced production of the type-1 cytokines interferon (IFN)-γ and interleukin (IL)-18, the pro-inflammatory cytokines IL-6, -8 and RANTES (regulated on activation, normal T-cell expressed and secreted), but not any of the type-2 cytokines IL-4, -5, -10 and -13. Induction of IL-6, -8 and RANTES, but not IFN-{gamma} or IL-18, were shown to be dependent on virus replication. PHA induced all except IL-12, -13, and -15. Induction of IFN-{gamma}, IL-6, -8, and RANTES was significantly increased in atopic children. Induction of both IFN-{gamma} andIL-4 increased in parallel in relation to age, with no change in the IFN-{gamma}:IL-4 ratio. These data are compatible with the hypothesis that immature type-1 immunity during early childhood plays a role in both respiratory syncytial virus bronchiolitis and in its relationship with atopy.

European Journal of Cancer (36)


http://www.sciencedirect.com/science/article/B6T68-3Y2G9FW-6/2/efe0dc7af6b5693ae6e2d14b1e833

Although human papillomaviruses (HPVs) have been found in many, but not all, tumours of the oral cavity, nose, pharynx and larynx, the true role of HPV in malignant tumours of the head and neck is still unclear. The presence of HPV DNA was investigated in 45 fresh squamous cell carcinoma (SCC) specimens and in 29 normal mucosa specimens collected from 45 primary laryngeal SCC patients. HPV DNA was detected using the polymerase chain reaction (PCR) with consensus primers that detect HPV types 6, 11, 16 and 18. 9 of the 45 patients (20%) were HPV positive; the presence of HPV was also detected in the corresponding normal laryngeal mucosa of four of the 29 specimens (14%). No statistically significant differences were found between the
presence of HPV DNA in normal specimens and in neoplastic mucosa specimens. No correlation was found between HPV DNA positive tumours and size, T classification, lymph node involvement and histological grading. This study adds further evidence suggesting a possible role of HPV DNA infection in laryngeal carcinogenesis.


http://www.sciencedirect.com/science/article/B6T68-3YDFY6M-P/2/daec4ff752923e76e911725e82773739

The development of therapy-induced drug resistance is still one of the most important therapeutic limitations. Nevertheless, an integrating view of the molecular mechanisms underlying resistance development in general is missing. In order to shed some light on the network of this resistance development, we established drug-resistant (doxorubicin (DX), methotrexate (MTX), cisplatin (cisPt), vincristine (Vin)) derivatives of six tumour cell lines (Jurkat, U937, HL60, DoHH-2, K562 and ARH77) of haematopoetic origin. Differential gene expression of drug-sensitive parental cell lines and the drug-resistant derivatives thereof was analysed by suppressive subtractive hybridisation. After dot blot screening for differential expression and sequencing of the cloned PCR fragments, differential expression was confirmed by Northern blot analysis. In an attempt to discriminate for differentially expressed genes only related to one or the other of the investigated drugs, the cDNAs of various resistant sublines (doxorubicin-, methotrexate-, cisplatin-resistant Jurkat cells) were pooled and compared with the sensitive parental cell line. In addition, cDNAs of the resistant derivatives of the different haematopoetic tumour cell lines were pooled and compared with the pooled cDNAs of the corresponding sensitive haematopoetic cell lines to eliminate cell line to cell line variations that were not related to drug resistance. As a result of this screening, the following genes showed a higher (at least 2-fold) or exclusive expression in the drug-resistant variants: serglycin, sorcin, BMPG (bone marrow proteoglycan gene) and PTI-1 (prostate-tumour-inducing gene 1). In addition, elevated expression of hsp90, previously found by our group to be upregulated in the drug-resistant colon carcinoma cell line LoVo H67P was found to be overexpressed in drug-resistant HL60 cells.


http://www.sciencedirect.com/science/article/B6T68-4CP0Y VH-4/2/6702d79174c49189297f53c4f9a4e15a

The purpose of this study was to demonstrate the effects of lycopene, the major tomato carotenoid, on the expression of the BRCA1 and BRCA2 genes in three breast tumour cell lines, MCF-7, HBL-100, MDA-MB-231 and the fibrocystic breast cell line MCF-10a. Flow cytometry analysis showed a G1/S phase cell cycle arrest after treatment of the cells with 10 [mu]M lycopene for 48 h. mRNA expression was studied by quantitative reverse transcription-polymerase chain reaction using the Taqman(R) method. We observed an increase of BRCA1 and BRCA2 mRNA in the oestrogen receptor (ER)-positive cell lines (MCF-7 and HBL-100), and a decrease (MDA-MB-231) or no change (MCF-10a) in the ER-negative cell lines. BRCA1 and BRCA2 proteins were quantified by perfusion affinity chromatography. No variation in their expression was observed. These preliminary results on the effects of lycopene on the expression of BRCA1 and BRCA2 oncosuppressor genes in breast cancer may reflect cross-talk between the oestrogen and retinoic acid receptor (RAR) pathways.
The aim of this study was to assess the expression of cytokine transcripts, reflecting the type of ongoing immune responses at the site of human papillomavirus (HPV) infection, in relation to the development of cervical neoplasia. To this end reverse transcription-polymerase chain reaction (RT-PCR) was performed for interferon (IFN)[gamma], interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12 (p35 and p40), and transforming growth factor (TGF[beta]1) in snap-frozen cervical biopsies, which were tested for the presence of high risk HPV DNA and histologically classified from normal to invasive carcinoma (n=40). IFN[gamma], IL-10 and IL-12 (p35 and p40) transcripts were found to be expressed at significantly lower frequencies in invasive carcinoma as compared with premalignant biopsies (P=0.006, P=0.007 and P=0.002, respectively). IFN[gamma] and IL-10 mRNA were associated with the presence of the IL-12 p35 and p40 transcripts (P=0.008 and P<0.00001, respectively). These results are consistent with a locally reduced cellular (type 1) immunity correlating with HPV-induced invasive cervical carcinoma.


http://www.sciencedirect.com/science/article/B6T68-4BNVS1W-2/2/bdccc1e5a4767cc72a706f712eba9a2e0

Previous studies have shown that activating mutations of c-KIT/PDGFRA, potential therapeutic targets for imatinib mesylate, are implicated in the pathophysiology of gastrointestinal stromal tumours (GISTs). In this study, GISTs from 37 patients enrolled in an European Organisation for Research and Treatment of Cancer (EORTC) phase I/II clinical study of imatinib were examined for mutations of c-KIT/PDGFRA in order to explore whether the mutational status of the tumour predicts the clinical response to therapy. Mutations were screened by denaturing high-pressure liquid chromatography (DHPLC) and characterised by bi-directional DNA sequencing. Activating mutations of c-KIT or PDGFRA were found in 29 (78%) and 2 (6%) GISTs, respectively. Most c-KIT mutations involved exon 11 (n=24; 83%), all but one being an in-frame deletion; no isolated point mutations were found. The other c-KIT mutations included exon 9 AY 502-503 duplication (n=4; 14%) and exon 13 Lys->Glu642 missense mutation (n=1; 3%). Two tumours with no detectable c-KIT mutations demonstrated PDGFRA Asp->Glu842 amino acid substitutions. Patients with GISTs harbouring exon 11 mutations were more likely to achieve a partial response (PR) on imatinib therapy (83%) than all of the others (23%). The overall survival and progression-free survival rates for the entire group at 106 weeks were 78.3% and 46.9%, respectively. Based on a Kaplan-Meier analysis, patients with GISTs harbouring c-KIT mutations had longer median survival times and were less likely to progress than the other patients. These findings indicate that the mutational status of the c-KIT/PDGFRA oncoproteins could be useful to predict the clinical response of patients imatinib therapy.

Polymerase chain reaction (PCR) products representative of the DNA sequence coding for the variable heavy (vH) and the variable light (VL) chains of an anti-MUCI mucin monoclonal antibody, C595, have been produced. These products were cloned, sequenced, and the primary amino acid sequences of the VH and VL regions deduced. The hypervariable complementarity determining regions (CDRs) and framework regions in the heavy and light chains were located, and homologies with canonical forms for the CDR loops L1, L2, L3, H1 and H2 were identified by database searching. The structure for the H3 loop was calculated directly. Computational molecular modelling was accomplished using the fully automated AbM package (Oxford Molecular, Oxford, U.K.). Energy minimisation was performed using the program InsightII (Biosym, San Diego, California, U.S.A.). The investigation provides a basis for the molecular analysis of the antigen binding site of the C595 antibody with the aim to identify key residues and interactions involved in the immune recognition of the C595 antibody defined epitope, which is expressed in the majority of breast and ovarian carcinomas.


This report describes an unusual clinical presentation of Li-Fraumeni syndrome. Family history revealed a mild aggregation of adult cancers in one generation, and an unusual clustering of brain tumours of early childhood in the following generation. In order to evaluate the genetic basis for cancer predisposition in this family, molecular genetic analysis for the occurrence of germline TP53 tumour suppressor gene mutations was performed on 12 siblings of two generations. Indirect mutation analysis was performed by the single-strand conformation polymorphism (SSCP) technique. Alterations were characterised by automated direct fluorescence sequencing analysis. Tumour material was also examined for p53 protein accumulation by immunohistochemistry. Initially, a TP53 gene germline missense mutation was detected in an 11-year-old kindred with acute myeloid leukaemia (AML) following intensive treatment of a brain tumour. In peripheral blood and bone marrow samples of this proband, a reduction to hemizygosity occurred. During AML treatment, detection of LOH of 17p was used as a marker for clonality and treatment control. The mutation was found to be inherited from the proband’s mother, who was diagnosed with breast cancer at the age of 48 years. Further, three siblings were carriers, and two are apparently healthy at the age of 21 and 23 years. Knowledge of germline mutations may allow accurate DNA-based carrier diagnosis which is of important clinical significance for treatment strategy and control. Furthermore, the occurrence of unaffected carriers in this family raises questions about appropriate methods of cancer surveillance and counselling for these people.

The aim of this study was to describe and characterise a founder mutation of the BRCA1 gene in western Sweden. Of 62 families screened for BRCA mutations, 24 had BRCA1 mutations and two had BRCA2 mutations. Tumours that occurred in family members were histologically reviewed and mutational status was analysed using archival paraffin-embedded tissues. The same BRCA1 mutation, 3171ins5, was found in 16 families who were clustered along the western coast of Sweden. Mutation analysis revealed a maternal linkage in 13 families and a paternal linkage in 3. There was complete agreement between mutation analysis results obtained from blood and archival tissues. The penetrance of breast or ovarian cancer by age 70 years was estimated to be between 59 and 93%. There were no differences in survivals between breast or ovarian cancer patients with the mutation and age-matched controls. Thus, a predominant BRCA1 gene founder mutation associated with a high risk of breast and ovarian cancer has been identified and found to occur in a restricted geographical area, thereby allowing timely and cost-effective mutation screening using blood samples or archival histological material.


http://www.sciencedirect.com/science/article/B6T68-47HSPGV-N/2/118c70b0aa76488dc8f1c49d4be5bd65

Individuals with an inherited predisposition to cancer development are at an increased risk of developing multiple tumours. Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common hereditary cancer syndromes and is estimated to account for approximately 2% of colorectal cancers. However, HNPCC individuals are at an increased risk of developing other tumour types such as cancers of the endometrium, urothelium and small intestine. We have utilised a population-based regional cancer registry to identify all patients with double primary colorectal cancers and at least one additional malignancy and characterised the tumour spectrum in this patient group. We subsequently selected those 47 individuals who had developed at least four malignancies, including two colorectal cancers, for studies of the tumour characteristics associated with HNPCC. In total, these individuals developed 209 tumours, 156 of which were successfully retrieved. Microsatellite instability (MSI), a phenomenon caused by defective mismatch-repair (MMR), was identified in 63/154 (41%) evaluable tumours with a MSI-high pattern in 59 and a MSI-low pattern in four tumours. All tumours were immunohistochemically stained for the MMR proteins MLH1 and MSH2, with loss of expression in 55/63 (87%) MSI tumours and in 2/89 (2%) microsatellite stable (MSS) tumours. This loss affected MLH1 in 24 tumours and MSH2 in 33 tumours. A concordant loss of expression for the same MMR protein in several tumours from the same individual, a pattern that strongly suggests an underlying germline MMR gene mutation, was found in 17/45 (38%) patients and affected MLH1 in 8 patients and MSH2 in 9 patients. We conclude that the development of multiple primary tumours, including synchronous or metachronous colorectal cancers, is associated with an increased frequency of MSI and loss of immunohistochemical expression of MLH1 and MSH2.


http://www.sciencedirect.com/science/article/B6T68-41FTRGV-K/2/6c4237b788729c0507a8495bb759307f

In tumour cells, replicative immortality is attained through stabilisation of telomeres by telomerase. Recent evidence suggests that telomerase plays an anti-apoptotic role. Since apoptosis is the primary mode of cell death induced by several drugs, telomerase could be involved in determining the chemosensitivity profile of tumour cells. We investigated whether
inhibition of telomerase activity through a hammerhead ribozyme targeting the RNA template of telomerase influences the susceptibility of human melanoma cells to a variety of anticancer agents (platinum compounds, taxanes, topoisomerase I inhibitors). The ribozyme sequence was inserted into an expression vector and the JR8 human melanoma cell line was transfected with it. The cell clones obtained showed a reduced telomerase activity. Growth inhibition curves generated after exposure of ribozyme-transfectant clones to individual drugs were superimposable to those obtained from parental cells. Moreover, telomerase inhibition did not promote apoptosis as a cellular response to drug treatment. Overall, our results indicate that downregulation of telomerase activity does not increase the sensitivity of melanoma cells to anticancer drugs.


http://www.sciencedirect.com/science/article/B6T68-3T6YGN1-1X/2/639b75b16c092c34d8fc9d8cb472aa3e

The aim of this study was to investigate the expression of p53 and bcl2 proteins in a series of 107 non-small cell lung cancers (NSCLC), and to relate such protein expression to neovascularisation and the expression of vascular endothelial growth factor (VEGF). Moreover, we analysed the prognostic impact of these biological parameters on overall survival, both in univariate and multivariate analyses. An inverse association was found between bcl2 expression and microvessel count (MVC; P=0.0004) and bcl2 and VEGF (P=0.007). In contrast, a significant association was found between p53 expression and MVC (P=0.03) and p53 and VEGF expression (P=0.04). In univariate analysis, nodal status (P<0.002), p53 (P=0.03) and VEGF expression (P<0.000001) significantly affected overall survival, but in multivariate analysis only MVC and VEGF expression retained their prognostic influence. Our results suggest that bcl2 and p53 possibly control the development of tumour angiogenesis in NSCLC, with putative mediation by VEGF. Moreover, the important influence of angiogenesis in the progression of NSCLC is further highlighted.


http://www.sciencedirect.com/science/article/B6T68-43PG3HB-K/2/fe512e61a4b51b4f0d632e7c231e3c09

Non-small cell lung cancer is associated with approximately 85% mortality due to its high metastatic potential. Therapeutic efforts have failed to produce a significant improvement in prognosis. In this situation, a better understanding of the key factors of metastasis may be useful for designing new molecular targets of therapy. In order to identify these factors, we compared the expression profiles of two subpopulations of an adenocarcinoma cell line with a high metastatic potential, PC9/f9 and PC9/f14, with the parent cell line, PC9, using a cDNA array. The expression of 15 genes was found to be significantly enhanced or reduced in the highly metastatic subpopulations. The expression of matrix metalloproteinase-2 (MMP-2), plasminogen activator inhibitor-1 (PAI-1) and interleukin-1 (IL-1[alpha]) were upregulated in the highly metastatic subpopulations, while the expression of carcinoembryonic antigen (CEA), caspase-5, Fas ligand, Prk/FNK, cyclin E, cyclin B1, Ki-67, proliferating cell nuclear antigen (PCNA), Smad4, macrophage proinflammatory human chemokine-3[alpha] (MIP-3[alpha])/LARC, Met and CD44 were downregulated. Data from the literature suggest that the altered expression of MMP-2, PAI-
IL-1[alpha], CEA, caspase-5, Fas ligand, Prk/FNK and Smad4 promotes the highly metastatic phenotype. The differential expression of these genes was confirmed by Northern blot analysis, standard reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR. This analysis in subpopulations of a lung cancer cell line indicated that the highly metastatic potential of lung cancer may be induced not by an alteration in the expression of a single gene, but by the accumulation of alterations in the expression of several genes involved in extracellular matrix (ECM) adhesion disruption, ECM degradation, escape from apoptosis, and resistance to transforming growth factor-[ss]1 (TGF-[ss]1). Strategies for inhibiting metastasis of pulmonary adenocarcinoma should be designed accordingly.


http://www.sciencedirect.com/science/article/B6T68-47VH05T-5/2/ab7949f637a58323c62cf1fbf32b7c2

Type 1 cytokines, such as interferon gamma (IFN[gamma]) and interleukin-2 (IL-2), increase T cell-mediated immune responses and are considered to be beneficial for antitumour immunity. Type 2 cytokines, such as IL-4, IL-5, and IL-10, inhibit Type 1 responses and promote humoral responses. We have previously reported an association between low intratumoral IFN[gamma] mRNA levels and poor clinical outcome in patients with invasive cervical carcinoma. In this study, by using quantitative polymerase chain reaction (PCR), we identified a group of cervical carcinoma patients with undetectable intratumoral T cell-derived cytokine mRNAs, as IFN[gamma], IL-4 and IL-17 expression could not be detected in 5, 25 and 8 of the 52 biopsies analysed, respectively. Global downregulation of Type 1 and Type 2 cytokines was observed in a subgroup of patients who more frequently presented advanced stage tumours. Biopsies of patients with no IFN[gamma] gene expression did not appear to be less infiltrated by T cells than control biopsies with measurable IFN[gamma] gene expression. These results clearly demonstrate that, in some clinical situations, the decrease in intratumoral Type 1 cytokines is not associated with a Type 2 polarisation, but rather reflects global deactivation of T cells at the tumour site. These data provide support for immunotherapy protocols designed to reverse the anergic state of T cells in cancer.


http://www.sciencedirect.com/science/article/B6T68-4C35SM2-D/2/796512f37e19ed1df27ae46fffc59946c

A significant proportion of cervical carcinomas show loss of major histocompatibility complex human leucocyte antigen (HLA) class I expression while upregulating HLA class II expression. These changes may have direct consequences for immune surveillance of the human papilloma virus (HPV) infection which is strongly associated with cervical malignancy. A relationship between changes in HLA expression and HPV infection may be evident in the evolution of premalignant disease. This immunohistological study of 104 colposcopic biopsies establishes that HLA class II expression occurs in a significant proportion of squamous epithelia showing histological evidence of wart virus infection and cervical intraepithelial neoplasia (CIN) I to III. In comparison, alteration of HLA class I expression in cervical premalignant lesions is rare. There is no correlation between the detection of high risk HPV DNA (types 16, 18, 31 and 33) by polymerase chain reaction (PCR) and the MHC class II phenotype of the lesion. This suggests that altered HLA class II expression is neither a consequence nor a prerequisite for HPV

http://www.sciencedirect.com/science/article/B6T68-3W316N0-14/2/2f6cc6592daf9663218e43b7ccef40e2

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein, which removes alkyl groups from the O6 atom of guanine residues. Tumour cells which lack MGMT are sensitive to cytostatic drugs such as dacarbazine (DTIC), whose active species bind to this site. To explore whether analyses of MGMT expression can be used as a predictive test for clinical sensitivity to DTIC in melanomas, we developed a method to assay MGMT mRNA levels in cells obtained by fine needle aspiration biopsies of metastases. cDNA was synthesised from mRNA prepared from biopsy material. Polymerase chain reaction was performed using primers complementary to MGMT cDNA and to [beta]-actin, which served as an internal control. Analyses of 44 biopsies from 35 patients showed a considerable variation in MGMT mRNA, with 15 samples (34%) lacking detectable mRNA. In 6 out of 8 patients in whom more than one tumour was analysed, separate metastases had different levels of MGMT mRNA. There was no correlation between MGMT activity studied by a biochemical assay and MGMT mRNA levels when these were compared in 10 surgical biopsies.


http://www.sciencedirect.com/science/article/B6T68-47GGV0W-F2/1/1cec791298cd68aa284f1b0a3d979705

Heparanase (hep) degrades heparan sulphate proteoglycans (HSPGs), which are the main components of the extracellular matrix. This process has been considered as the first step of tumour invasion or metastasis. However, HSPGs play an important role in signal transduction. Thus, the degradation of HSPGs by hep may suppress tumour cell growth. In the present study, we investigated the clinicopathological importance of enhanced hep mRNA expression in 48 hepatocellular carcinomas (HCCs) and in 48 non-cancerous liver samples obtained from the same patients by quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). Spontaneous apoptosis in the hepatocytes was evaluated by immunohistochemistry. The relative hep mRNA expression levels were described as hep/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ratios. The hep mRNA levels of HCCs were significantly lower than those of non-cancerous livers (PHep mRNA levels decreased with increasing liver fibrosis. A significant positive correlation between hep gene expression and spontaneous apoptosis was detected. Hep expression in the tumours did not correlate with tumour differentiation or with tumour stage. However, low hep gene expression was associated with a poor disease-free survival of the patients. Thus, hep gene expression may play an important role in programmed cell death and this gene expression may be lost during the malignant transformation of hepatocytes.

Several tumour-forming cell lines are known to overproduce the lysosomal cysteine peptidase cathepsin L. We have used an antisense approach to investigate whether inhibition of cathepsin L overexpression in two malignant cell lines (myeloma SP cells and L cells) reduces their tumorigenic potential. Two different cDNA fragments of murine cathepsin L were inserted in the antisense direction into the pcDNA3 vector, and SP and L cells were stably transfected with these plasmid constructs. Several of the selected clones expressing the antisense transcript showed specific reduction of the mRNA level and the intracellular activity of cathepsin L, and a greatly diminished amount of secreted procathepsin L. When tested in Balb/c nu/nu mice, the cell lines with low cathepsin L activity exhibited a significantly decreased potential for tumour growth when compared with control cells expressing wild-type levels of cathepsin L activity. This observation suggests that cathepsin L is a critical factor in tumour growth.


Using four cell lines including drug-sensitive K562/Parent cells, P-glycoprotein (Pgp)-mediated multidrug resistant (MDR) K562/VCR, K562/ADR and revertant K562/ADR-R ceUs, two fluorescent agents, Fluo-3 and rhodamine-123 (Rh-123), were compared as indicators in a functional assay of MDR. Cells were incubated with 4 [mu]M Fluo-3 or 1 [mu]M Rh-123 for 45 min and then the intracellular accumulation of the agent was measured using a flow cytometer. Verapamil (20 [mu]M) or cepharanthine (bicoclaurine alkaloid, 10 [mu]M) was added just before the fluorescent agents. Efflux patterns were also studied 60 min after incubation with or without verapamil and cepharanthine. Increased intracellular accumulation and a delayed efflux pattern of Fluo-3 by verapamil and cepharanthine were demonstrated in multidrug resistant K562/VCR and K562/ADR cells, indicating that Fluo-3 is another good indicator of MDR. However, a similar, but lower, increase in uptake and a delayed efflux pattern of Fluo-3 by verapamil and cepharanthine were also demonstrated even in Pgp-non-overexpressed K562/Parent cells. In contrast, accumulation of Rh-123 was not affected by verapamil and cepharanthine. To further study the Pgp dependency of Fluo-3, another cell line, K562/NC16 expressing minimum MDR1 mRNA, was cloned. Increased uptake and a delayed efflux pattern of Fluo-3, but not Rh-123, with verapamil or cepharanthine were again demonstrated in K562/NC16 cells, indicating that intracellular accumulation of Fluo-3 may be nonspecifically influenced by verapamil and cepharanthine at very low levels of Pgp-related MDR, while the influx and efflux patterns of Rh-123 may be specifically affected by Pgp overexpression.


At least 10% of all ovarian cancers are estimated to have a hereditary background. Hereditary breast-ovarian cancer (HBOC) due to mutations in the BRCA genes is a major cause of hereditary ovarian cancer, although its frequency and relationship to age and family history in
unselected series of ovarian cancers is not completely known. We report here the results of a full mutational screening analysis for germ line BRCA1 and BRCA2 mutations in 161 patients with invasive epithelial ovarian carcinomas. Age at diagnosis ranged from 22 to 82 years (mean 59 years). Deleterious (frame-shift, nonsense and missense) mutations were detected in 13/161 (8%) of the patients and affected BRCA1 in 12 cases and BRCA2 in one case. Four additional missense variants (one in BRCA1 and three in BRCA2) with a possible association with an increased risk ovarian cancer were revealed, resulting in a total frequency of BRCA gene alterations of 17/161 (11%). The 13 patients with deleterious mutations had a mean age of 57 years (range 41-76 years) and only three of these patients were below 50 years of age. A family history of at least one breast cancer and/or ovarian cancer was reported in all but 1 of the patients with BRCA mutations compared with only 24% of patients without mutations. Our findings in this prospective study confirm approximately 1 in 10 patients with ovarian cancer carry a germ line BRCA gene mutation associated with HBOC, and also indicate that a large number of these patients are over 50 years of age at diagnosis.


http://www.sciencedirect.com/science/article/B6T68-46JYK12-1/2/c554e317b09b0ee97069a6a6de01d8c3

Overexpression of the epidermal growth factor receptor (EGFR) often correlates with an aggressive tumour phenotype and poor prognosis. To examine the relevance of EGFR in colorectal cancer, we determined the expression of EGFR protein in 249 colorectal adenocarcinomas and 42 lymph node metastases using immunohistochemistry. Moreover, we investigated a (CA)n dinucleotide repeat polymorphism of the EGFR gene in a subset of 114 tumours. High levels of EGFR protein were observed in 123/249 (49.4%) samples. EGFR expression in colorectal carcinomas correlated with differentiation grade (P=0.014). However, there were no associations with Dukes' stage, site, patient age or gender. EGFR protein expression did not influence survival in this colorectal cancer patient cohort (P[ges]0.05). Expression was not identical in paired colorectal tumours and lymph node metastases, with only 17/42 (40.5%) samples showing equivalent EGFR levels (P>0.05). The distribution of the (CA)n dinucleotide repeat alleles in colorectal adenocarcinomas was not associated with EGFR protein expression (P>0.05). These results indicate that while EGFR overexpression is a common event in colorectal carcinogenesis, it does not influence patient prognosis.


http://www.sciencedirect.com/science/article/B6T68-3YWYBW-G/2/524952f0e2f9d949e93f30b40b13f

A number of genes, including IGF2 and H19, are normally imprinted with preferential expression of the paternal or maternal allele, respectively. Loss of imprinting (LOI) of IGF2 and H19 is found in a number of tumours, suggesting that LOI of IGF2 and/or H19 may play an important role in tumorigenesis. The IGF2 gene codes for a fetal growth factor and the H19 gene is likely to act as an RNA with an antitumour effect. We investigated the imprinting status of IGF2 and H19 in human meningiomas. The normally imprinted IGF2 gene lacks imprint in the leptomeninges and choroid plexus of the brain. To examine the imprinting status of IGF2 and H19 in human meningiomas we used the Apal polymorphism in exon 9 for the IGF2 gene and the AluI polymorphism in exon 5 for the H19 gene. In total, 24 meningiomas of WHO grade I, II and III were analysed. 15 meningiomas (63%) were informative for the Apal polymorphism in the IGF2
Monoallelic expression (MAE) for IGF2 was found in 11 out of 15 tumours (73%) which is in contrast to the lack of imprinting status of IGF2 in leptomeninges. Ten cases (42%) were heterozygous for the H19 gene and biallelic expression was found in 3 out of 10 meningiomas (30%). These results indicate that modulation of the imprinting status of IGF2 and H19 may play an important role for the development of meningiomas.


http://www.sciencedirect.com/science/article/B6T68-3WNMG0K-C/2/45cd43efa09304dae88e3e4b7d1877ca

We analysed microsatellite instability (MSI) in a consecutive series of 165 rectal carcinomas. Data on a personal and/or family history of cancer were collected from all patients and revealed metachronous cancer in 9 patients, 2 of whom had developed colorectal cancer, and a suspected familial aggregation of colorectal cancer in three families. Only three of the 165 (2%) rectal cancers showed MSI. The patients whose tumours displayed MSI had clinical histories suggesting hereditary cancer—a family history of colorectal cancer and/or synchronous colorectal cancers. Denaturing gradient gel (DGGE) analysis was used to screen the MSI+ patients for mutations in the hMLH1 and hMSH2 genes and revealed two new germline mutations; a 1 bp deletion in exon 10 of hMSH2 creating a premature stop-codon and a splice donor site mutation in intron 16 of hMLH1. Considering colorectal carcinomas as a group, MSI has been reported to occur in approximately 10-20% of the tumours and thus can not, per se be used for clinical detection of hereditary tumours. This study shows, however, that MSI is rare in rectal carcinomas and when present strongly suggests a hereditary predisposition for colorectal cancer development.


http://www.sciencedirect.com/science/article/B6T68-4CDHRH3-B9/2/a13207966e8c006c993f081b0e88c

Mutations in ras genes have been found in the DNA of numerous cancer types including melanomas, but the expression of these mutations in melanomas has not yet been addressed. We have used the polymerase chain reaction (PCR) and allele-specific restriction analysis (ASRA) to determine the frequency of expressed N-ras mutations on 25 short-term melanoma tissue culture samples. N-ras cDNA generated using reverse transcriptase from whole cells was used as the PCR template. 14 secondary melanoma cultures that varied in differentiation patterns were analysed. Only 2 were found to express N-ras mutations; in both, the mutation was localised to one of the first two positions of the 61st codon of N-ras. These tumour lines, KMI-M8412a and KMI-M8412b, were established from separate tumour deposits in the same patient. Codons 12 and 13 were found to be free of mutations in all of the lines studied. 8 primary melanomas and 3 unclassified skin lesions were also analysed and found free of N-ras mutations. These results suggest that N-ras may not play such an important role in melanoma tumorigenesis as is speculated by others.

Pejovic, T., D. Ladner, et al. (2004). "Somatic D-loop mitochondrial DNA mutations are frequent in uterine
The mitochondria plays a role in apoptosis. Its genome is also more susceptible to mutations because of high levels of reactive oxygen species and limited repair mechanisms. The D-loop of mitochondrial DNA (mtDNA) contains essential transcription and replication elements, and mutations in this region might alter the rate of DNA replication. We examined genetic alterations in the D-loop region of mtDNA in uterine serous carcinoma (USC) samples and their paired normal adjacent endometrium. DNA was extracted after laser-capture microdissection of paraffin-embedded tissues from eight patients with USC. The entire D-loop genome was amplified using nine pairs of overlapping primers. Denatured polymerase chain reaction (PCR) products were subjected to single-strand conformation polymorphism (SSCP) analysis. Somatic mtDNA alterations were detected in five tumours (63%). Our study indicates that mtDNA D-loop sequence alterations occur at a high frequency in USC suggesting that mtDNA mutations may play a role in the development of USC.


The correlation between inactivation of the TP53 gene through mutation or the presence of high-risk human papillomavirus (HPV) DNA and intrinsic paclitaxel sensitivity was studied in 27 gynaecological cancer cell lines. IC50 values, as a measure of drug sensitivity, were determined using a 96-well clonogenic assay. TP53 mutations were investigated with polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and direct DNA sequencing. HPV status was studied with PCR using HPV consensus primers. TP53 mutations were found in 7/11 vulvar SCC cell lines. Only 2/9 endometrial and 1/7 ovarian cancer cell lines carried TP53 mutations. One vulvar and one endometrial cancer cell line were HPV-positive; both carrying HPV type-16 DNA. Thus, TP53 was functionally normal in 3/11 vulvar, 6/9 endometrial and 6/7 ovarian cancer cell lines. The IC50 values for paclitaxel were 0.60-2.9, 0.49-2.3 and 0.40-3.4 nM in the vulvar, endometrial and ovarian cancer cell lines, respectively. No correlation could be demonstrated between inactivation of the TP53 gene and paclitaxel sensitivity in vitro; the cell lines were evaluated as one group or according to their anatomical origin or histology. Previous reports have given inconclusive results, partly due to the cell types used, i.e. normal, cancerous or transformed cells. Our results support the view that paclitaxel sensitivity of tumour-derived cancer cell lines is not related to the TP53 status.


Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been widely used as a control RNA in Northern blotting and in reverse transcriptase-polymerase chain reaction (RT-PCR) analyses. We investigated the expression of GAPDH in a large series of primary breast cancers and in
MCF7 human mammary epithelial breast cancer cells treated with oestradiol. The expression of GAPDH was quantified by a real-time one-step RT-PCR assay, based upon the 5’ nuclease activity of Taq polymerase using an Abi Prism 7700 Sequence Detector System (Perkin Elmer, France). Using the Spearman test, GAPDH expression was found to correlate inversely with the age of the patients at diagnosis ($P=0.003$; $r=-0.147$), oestradiol receptors (ER) ($Pr=-0.327$) and progesterone receptors (PgR) ($Pr=-0.206$). A positive correlation was observed between GAPDH expression and the histo-prognostic grading (HPG) ($Pr=0.344$). Moreover, the overall survival (OS) and the relapse-free survival (RFS) were significantly reduced in patients whose tumours showed an enhanced level of GAPDH expression (OS, $P=0.046$; RFS, $P=0.021$). Multivariate analyses demonstrated that GAPDH was not an independent prognostic factor. Finally, in MCF7 cells treated with oestradiol, a statistically significant dose-dependent increase in GAPDH expression was observed. These results show that GAPDH expression is associated with breast cancer cell proliferation and with the aggressiveness of tumours. The present study demonstrates that, in cancer, the use of GAPDH gene expression should not be used as a control RNA.


http://www.sciencedirect.com/science/article/B6T68-48KVD9M-8/2/afe4d8fc8ee9ae00b3cf56151118640

In this study, we investigated whether (a) carcinoembryonic antigen (CEA), cytokeratin-20 (CK-20) and guanylyl cyclase C (GCC) are clinically useful markers for the molecular detection of submicroscopic metastases in colorectal cancer (CRC) and (b) whether overexpression of CEA, CK-20 and GCC can be reliably detected in formalin-fixed, paraffin-embedded tissues as well as frozen lymph nodes. We studied 175 frozen lymph nodes and 158 formalin-fixed, paraffin-embedded lymph nodes from 28 cases of CRC. CEA or CK-20 or GCC-specific polymerase chain reaction (PCR) was carried out on mRNA transcripts extracted from the nodal tissues. Ten out of 11 Dukes’ B CRC cases had detectable CEA and CK-20 while 6 out of 11 Dukes’ B CRC cases had detectable GCC. In general, the difference of re-staged cases when comparing frozen and paraffin-embedded samples was marked; the only statistically significant correlation between frozen and paraffin tissue was for the CEA marker. Our results indicated a high incidence (>50%) of detecting micrometastases in histologically-negative lymph nodes at the molecular level.


http://www.sciencedirect.com/science/article/B6T68-4FJTNXV-1/2/eeaa0a3b42d469f230fedef319f562b4

Hyalinizing trabecular tumour (HTT) of the thyroid is a neoplasm of follicular derivation that shares several morphological similarities with papillary thyroid carcinoma (PTC). In this study, we investigated the prevalence of B-raf point mutations, RET/PTC rearrangements and N-ras point mutations in a large HTT series (28 samples). Twenty benign thyroid lesions and 10 PTC served as control cases. A high (47%) prevalence of RET/PTC rearrangements was found in HTT. By contrast, neither B-raf nor N-ras mutations were found in HTT. These findings suggest that, although RET/PTC, N-ras, and B-raf proteins may act along the same signalling cascade, the biological and morphological outcome of their oncogenic activation is not completely overlapping. Thus, in clinical practice, the detection of B-raf mutations in a thyroid follicular tumour may prove to be a valuable tool, supplementing histological examination, and allowing a differential
diagnosis between PTC and HTT.


http://www.sciencedirect.com/science/article/B6T68-4B84SKM-3/2/20a14be8cfb590beb4dd9d820b0bbcb3

The aim of this study was to determine whether nucleic acids are detectable in cell-free bronchial lavage supernatants, and whether it is possible to find alterations in this DNA and RNA of genes known to be present in lung tumour cells. DNA was isolated from cell-free lavage supernatants from 30 and RNA from 25 lung cancer patients. The DNA was examined for microsatellite alterations (MA) and the RNA analysed for the expression of seven tumour-associated genes. Intact DNA and mRNA could be isolated from all cell-free bronchial lavage supernatants. MA were found in lavage supernatants of 12/30 patients and in lavage cells of 6/30 patients. Altogether alterations were found in lavage supernatants of 14/30 patients and in lavage cells of 6/30 patients. Thus, we could demonstrate, for the first time, that it is possible to isolate intact DNA and RNA from cell-free bronchial lavage supernatants. Their quantity and quality is sufficient for further amplification by polymerase chain reaction (PCR)/reverse transcriptase (RT)-PCR. Altogether, tumour-associated changes were detected in DNA samples from 47% of the patients and in RNA samples from all of the patients analysed.


http://www.sciencedirect.com/science/article/B6T68-3YS90M5-B/2/3588ee870912e234dd799de0831021cd

The aim of the study was to clarify the role of telomerase component genes in hepatocarcinogenesis and to examine both the relationship between the expression of telomerase component genes and histological differentiation in hepatocellular carcinoma (HCC) and the relationship between expression levels of telomerase component genes and telomerase activity in HCCs. Telomerase is a ribonucleoprotein enzyme composed of a template RNA and several proteins. Recently, three such telomerase component genes have been identified: human telomerase reverse transcriptase (hTERT); human telomerase RNA component (hTERC); and telomerase-associated protein 1 (TEP1). The expression of these components was evaluated in 34 HCCs and 24 non-cancerous liver tissues by reverse transcriptase-polymerase chain reaction (RT-PCR). Expression of hTERT mRNA was detected in most HCCs, but not in the non-cancerous tissues (PPP<0.01). These results suggest that telomerase reactivation during hepatocarcinogenesis might be regulated by only hTERT and an increase in telomerase activity level in tumour progression might be regulated by both hTERT and hTERC.


http://www.sciencedirect.com/science/article/B6T68-41FTRGV-9/2/7f11dc9e08d5858bb75ef8b17e5b1d09
Breast cancer in young women is uncommon and often presents with unfavourable biopathological features. Although early age at onset could suggest a genetic susceptibility to cancer, the appropriateness of BRCA1 testing for women with early-onset breast cancer and modest family history (FH) is controversial. 40 Women diagnosed with breast cancer at the age of 35 years or less, unselected for FH, were screened for germ line BRCA1 mutations by automated sequencing of exons 2, 5, 6, 11, 13 and 20. Overall, deleterious mutations were evidenced in 6 (15%) patients. With regard to FH, mutations were detected in 14%, 11% and 29% of women with none, weak and strong FH, respectively. Large tumour size, grade 3, lack of oestrogen receptors and high proliferation rate were significantly more common in mutation carriers (MC). Our data support both the appropriateness of testing young breast cancer patients and the frequency of unfavourable features in BRCA1-related breast cancer. It is hypothesised that BRCA1 mutations partially justify the high rate of aggressive breast cancer in young patients and that combining age and breast cancer phenotype could help to identify probable MC.


http://www.sciencedirect.com/science/article/B6T68-4D4CYMW-2/2/eac021d4e3c37b94b717d707a8ee375fe

The relative contribution of promoter hypermethylation and aberrant splicing to the inactivation of the fragile histidine triad (FHIT) gene is unclear. Using genetic and epigenetic analyses, the current investigation examines the loss of protein and mRNA expression, and 5'CpG hypermethylation and allelic imbalance of the FHIT gene in a series of 129 non-small cell lung cancer (NSCLC) samples, in parallel with clinicopathological analyses. We found that 50% of NSCLC patients had aberrant protein expression, which was more frequent in squamous cell carcinomas (SQ) (69%) than in adenocarcinomas (AD) (28%) (P FHIT was identified in 31% of patients. Abnormally-sized FHIT transcripts were also observed in 24% of patients and were attributed to various exonic deletions, mainly in the region of exons 4-8. Allelic imbalance of the FHIT locus and its correlation with the status of Fhit expression, 5'CpG hypermethylation, and aberrant splicing, indicated that biallelic inactivation of Fhit expression could be induced by 5'CpG hypermethylation of one allele and alternative splicing in the other allele. Moreover, an 83% concordance in the methylation status of FHIT was demonstrated between 12 samples of bronchial precancerous lesions taken before surgery and their matched resected tumours. Our data suggest that FHIT 5'CpG hypermethylation and splicing alterations are both predominant mechanisms involved in the aberrant expression of the FHIT gene, and that FHIT 5'CpG methylation may be potentially used as a supplemental detection marker for NSCLC.


http://www.sciencedirect.com/science/article/B6T68-3S6MN5H-C/2/0a3d1e4392dfe6d3261b7e70e7b1fe0

Dihydropyrimidine dehydrogenase (DPD) is responsible for the breakdown of the widely used antineoplastic agent 5-fluorouracil (5-FU), thereby limiting the efficacy of the therapy. It has been suggested that patients suffering from 5-FU toxicides due to a low activity of DPD are genotypically heterozygous for a mutant allele of the gene encoding DPD. In this study we investigated the cDNA and a genomic region of the DPD gene of a cancer patient experiencing severe toxicity following 5-FU treatment for the presence of mutations. Although normal activity of
DPD was observed in fibroblasts, the DPD activity in leucocytes of the cancer patient proved to be in the heterozygous range. Analysis of the DPD cDNA showed heterozygosity for a 165 bp deletion that results from exon skipping. Sequence analysis of the genomic region encompassing the skipped exon showed that the tumour patient was heterozygous for a G ->A point mutation in the invariant GT splice donor sequence in the intron downstream of the skipped exon. So far, the G->A point mutation has also been found in 8 out of 11 patients suffering from a complete deficiency of DPD. Considering the frequent use of 5-FU in the treatment of cancer patients, the severe 5-FU-related toxicities in patients with a low activity of DPD and the high frequency of the G->A mutation in DPD deficient patients, analysis of the DPD activity and screening for the G->A mutation should be routinely carried out prior to the start of the treatment with 5-FU.


http://www.sciencedirect.com/science/article/B6T68-3WH6334-5/2/bbfeb3d8ccfb25eb94d295479ed72f9f

The patched/hedgehog/smoothened signalling pathway has been implicated in the development of sporadic tumours associated with the naevoid basal cell carcinoma (Gorlin) syndrome (NBCCS). Mutations in sporadic basal cell carcinomas (BCCs) of the skin and medulloblastomas have been found in genes encoding all three proteins of the pathway. A substantial proportion of breast carcinomas has recently been suggested to contain missense mutations in the human patched (PTCH) and sonic hedgehog (SHH) homologues. However, an independent study showed that the implicated mutation in SHH (H133Y) was absent in a large number of BCCs, medulloblastomas, breast, ovary and colorectal tumours. We searched for the H133Y SHH mutation in 84 primary breast carcinomas, but did not detect this change in any sample. In addition, a subset of 45 primary breast tumours was analysed for mutations in the PTCH coding region and 48 samples in previously implicated exons of human smoothened, but no mutations were found. Although our results do not exclude the presence of clonal alterations of these genes in a small proportion of breast carcinomas, these data do not support the existence of frequent mutations in genes encoding major protein partners of this signalling pathway. The absence of nucleotide changes in PTCH may point to another linked gene in the chromosome region 9q22-q23, previously suggested to contain a breast cancer susceptibility gene.


http://www.sciencedirect.com/science/article/B6T68-49979VC-D/2/241269c2a1cfe0e7a8cece3e1e1d9fe

Epigenetic silencing of the p16 and p15 genes by promoter methylation are commonly observed in human epithelial malignancies, including head and neck squamous cell carcinomas (HNSCC). In this study, a methylation-specific polymerase chain reaction (MSP) was used to evaluate the methylation status of the p16 and p15 genes in 73 HNSCC surgical specimens. p16 and p15 gene methylation was also examined in 29 paired metastatic lymph nodes and 29 paired histologically, normal resection margin mucosae. The quantity of cell-free methylated p16 and p15 DNA in the plasma samples of 20 HNSCC patients and 24 healthy controls was also examined using a fluorescence-based real-time PCR assay. The frequencies of p16 and p15 methylation in the primary tumour were 49% and 60%, respectively. Concordant methylation of p16 and p15 in tumour samples and metastatic lymph nodes was found in 59 and 38% of cases,
respectively. A significantly higher prevalence of p15 methylation was found in histologically-
normal surgical margin epithelia of HNSCC patients with chronic smoking and drinking habits
compared with non-smokers and non-drinkers. In addition, methylated p16 and p15 DNA levels
were significantly higher in the plasma of HNSCC patients (mean 56 copies/ml plasma and 65
copies/ml plasma, respectively) compared with normal controls (mean 6 copies/ml plasma and 16
copies/ml plasma, respectively). In conclusion, promoter methylation of the p16 and p15 genes is
involved in the pathogenesis of HNSCC and may be related to chronic smoking and drinking. The
differential levels of methylated p16 and p15 DNA in plasma might be potential useful markers in
screening high-risk populations for early HNSCC and monitoring their treatment response.

Xu, C. F., J. Greenman, et al. (1998). "Truncated TSG101 transcripts are present in peripheral blood from
both familial breast cancer patients and controls." European Journal of Cancer 34(7): 1077.

http://www.sciencedirect.com/science/article/B6T68-3THY0JD-
T/2/fbdfo980ed6a5fec5d0f2e0af696d89

TSG101 is a recently identified putative tumour suppressor gene which has been implicated in
human breast cancer. To address whether germline disruption of TSG101 predisposes
individuals to this disease, we analysed genomic DNA and mRNA isolated from peripheral blood
from 20 familial breast cancer cases. No evidence of large intragenic insertions/deletions or point
mutations in TSG101 was found by Southern blot analysis and sequence analysis of the entire
coding region. However, in 11 of 20 samples, 'aberrant' transcripts were detected. Sequence
analysis suggested that these variants were generated by the use of different cryptic splicing
sites. Such alternative/aberrant splicing events were not restricted to cancer patients, but were
also detected in peripheral blood of non-cancer patients and in normal tissues.

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137.

http://www.sciencedirect.com/science/article/B7GGS-4C06G66-
8/2/d2df3e2c17e4c16f69e9547926eaf564

The polymerase chain reaction was used to examine paraffin-embedded tissues of 37
nasopharyngeal carcinomas (NPC) for Epstein-Barr virus (EBV) genomic sequences. EBV DNA
was found in 2/14 keratinising squamous cell (WHO 1) carcinomas and in all of 23 non-
keratinising and undifferentiated (WHO 2 and 3) NPC. The study confirms the infrequent
association of keratinising NPC and EBV, in contrast with the 100% association of the less
differentiated NPCs and the virus. The results may indicate a different carcinogenesis for the
WHO 1 NPC subtype.
Biomechanical forces are major epigenetic factors that determine the form and differentiation of skeletal tissues, and may be transduced through cell adhesion to the intracellular biochemical signaling pathway. To test the hypothesis that stepwise stretching is translated to molecular signals during early chondrogenesis, we developed a culture system to study the proliferation and differentiation of chondrocytes. Rat embryonic day-12 limb buds were microdissected and dissociated into cells, which were then micromass cultured on a silicone membrane and maintained for up to 7 days. Stepwise-increased stretching was applied to the silicone membrane, which exerted shearing stress on the cultures on day 4 after the initiation of chondrogenesis. Under stretched conditions, type II collagen expression was significantly inhibited by 44% on day 1 and by 67% on day 2, and this difference in type II collagen reached 80% after 3 days of culture. Accumulation of type II collagen protein and the size of the chondrogenic nodules had decreased by 50% on day 3. On the other hand, expression of the non-chondrogenic marker fibronectin was significantly up-regulated by 1.8-fold on day 3, while the up-regulation of type I collagen was minimal, even by day 3. The down-regulation in the expression of chondrogenic markers was completely recovered when cell-extracellular matrix attachment was inhibited by Gly-Arg-Gly-Asp-Ser-Pro-Lys peptide or by the application of blocking antibodies for [alpha]2, [alpha]5 or [beta]1 integrins. We conclude that shearing stress generated by stepwise stretching inhibits chondrogenesis through integrins, and propose that signal transduction from biomechanical stimuli may be mediated by cell-extracellular matrix adhesion.


Background: Angiotensin II (Ang II) is a potent vasoconstrictor and a deleterious factor in cardiovascular pathophysiology. Ang II receptor blockers (ARBs) have recently been introduced into clinical practice for treatment of hypertension and congestive heart failure. Aims: This study was undertaken to evaluate the inhibitory effects of ARBs on vasoconstriction in humans. Methods: Vasomotor tone was analyzed in endothelium denuded, human coronary artery (HCA) segments. Ang II type 1 (AT1) and type 2 (AT2) receptor mRNA expression was examined by reverse transcriptase-polymerase chain reaction (RT-PCR). Results: Ang II was a potent vasoconstrictor (pEC50=7.7). At 1 nM of the AT1 receptor antagonists, candesartan and valsartan, the maximum contraction was depressed to 57 and 50% of Ang II, respectively,
indicating insurmountability. Although generally considered surmountable, the presence of 100 nM losartan elicited a depression of the Ang II response to 32%. Its active metabolite, EXP 3174 (1 nM), abolished the Ang II contraction. The AT1 receptor antagonists had the following order of blocking effect; EXP 3174>candesartan=valsartan>losartan. The AT2 receptor antagonist, PD 123319 (100 nM) significantly attenuated the Ang II contraction (Emax=62% of Ang II). RT-PCR of HCA smooth muscle cells demonstrated expression of both AT1 and AT2 receptor mRNA. Conclusions: Ang II contraction in HCA is mediated mainly by AT1 but also involves AT2 receptors. The active metabolite of losartan, EXP 3174, is the most efficacious AT1 receptor antagonist in HCA.

European Journal of Obstetrics & Gynecology and Reproductive Biology  (5)


http://www.sciencedirect.com/science/article/B6T69-4CGNT17-3/2/1ae92d677c728b6f0348a41e5298bd18

Objective: To investigate the presence of mutations in the open reading frame (ORF), as well as on the 5’ and 3’, flanking regions of the SRY gene in patients with mixed gonadal dysgenesis (MGD) or with Turner syndrome (TS) and Y mosaicism. Study design: We studied 13 patients with MGD and three patients with TS and Y mosaicism. DNA was isolated from blood leukocytes for subsequent polymerase chain reaction (PCR) and direct sequencing were performed in the ORF, as well as from the 5’ and 3’ flanking regions of the SRY gene. Results: No mutations were present in any of the patients studied. Conclusion: The absence of mutations in these regions indicated that mutations were an unlikely cause of MGD or TS with Y mosaicism and suggested that there are others genes playing an important role in sex development.


http://www.sciencedirect.com/science/article/B6T69-46XHT8B-3/2/8c35c302d3ec0c8df3f6d57d25e43b52

Objective: To investigate the correlation of the androgen receptor gene microsatellite polymorphism (CAG trinucleotide repeat polymorphism on exon 1) with bone mineral density and their relationship to osteoporosis in postmenopausal women. Study design: A number of 168 of 477 postmenopausal women were randomly recruited. The androgen receptor gene microsatellite polymorphism was determined using polymerase chain reaction-based microsatellite analysis. Bone mineral density of the lumbar spine and proximal femur was measured using dual-energy X-ray absorptiometry. Results: The AR genotype was classified from "9" to "32" according to the number of CAG trinucleotide repeats they contained to represent "signposts". After adjustment for potential confounding factors such as age, height, weight, years since menopause, and daily calcium intake, subjects with genotype 20+ (n=64) had lower bone mineral density values and a significantly greater risk for osteoporosis (OR 4.2, 95% CI 1.0-17.2) when compared with subjects
with genotype 20- (n=104) at the femoral neck. Conclusion: The present study suggests that the androgen receptor gene microsatellite polymorphism may be a candidate genetic marker for risk of osteoporosis in postmenopausal women.


http://www.sciencedirect.com/science/article/B6T69-43F77MR-7/2/63ee83ca1fcb2114da0d2f6f9d044fa4

Objective: To investigate the maturation of the paracrine system's endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), endothelin-1 (ET-1) and adrenomedullin (AM) in human placenta during the 2nd and 3rd trimester of pregnancy. Study design: Placental tissue from 14 healthy women with normal pregnancy and from 13 patients giving birth to premature infants following premature labor was obtained. Messenger RNA expression was determined using quantitative TaqMan real-time PCR. Results: Placental eNOS/GAPDH and ET-1/GAPDH mRNA expression significantly increased as a function of gestational age (r=0.63, Pr=0.53, P=0.007, respectively). There was no change in gene expression of neither iNOS nor AM mRNA/GAPDH during gestation (r=0.02, P=0.75 and r=0.001, P=0.99, respectively). Conclusion: There is a maturation of eNOS and ET-1 in human placenta with gestation reflecting developmental changes of important paracrine endothelial and trophoblastic regulators. AM and iNOS show no maturation during pregnancy.


http://www.sciencedirect.com/science/article/B6T69-44X87SS-F/2/6696122457fa61e6d2e2b62684b0560

Objective: The human placenta expresses a variety of vasoactive substances and neuropeptides, which play an important role in the regulation of placental blood flow in both the maternal and foetal compartment and are therefore of critical importance for foetal growth and development. Our study was planned to examine placental mRNA amounts of vasodilatory adrenomedullin (AM), calcitonin gene-related peptide (CGRP) and their receptors (AM-R and CGRP-R) in preeclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets). These are severe maternal conditions leading to an altered uteroplacental and fetoplacental perfusion and a higher risk for foetal growth retardation, premature delivery, infant mortality, and even maternal death. Study Design: We included 17 patients with preeclampsia, four women with HELLP syndrome and 34 controls. After delivery, the mRNA levels of AM, AM-R, CGRP, CGRP-R, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and [beta]-actin were measured in placental villi and chorionic plates using quantitative real-time PCR. Results: AM/[beta]-actin and AM/GAPDH mRNA ratios were significantly lower in placental villi in preeclampsia than in controls (PPConclusion: Our data show a reduction of AM and CGRP mRNAs in contrast to unchanged mRNA levels of their receptors in placenta specimens of women with preeclampsia or HELLP syndrome.

Maul, H., S. Nagel, et al. (2002). "Messenger ribonucleic acid levels of interleukin-1[beta], interleukin-6
and interleukin-8 in the lower uterine segment increased significantly at final cervical dilatation during term parturition, while those of tumor necrosis factor [alpha] remained unchanged." European Journal of Obstetrics & Gynecology and Reproductive Biology 102(2): 143.

http://www.sciencedirect.com/science/article/B6T69-44TD2KX-5/2/a5bdac7a07253f58e2b6f05a123b0f14

Objective: To investigate the content of tumor necrosis factor [alpha], interleukin-1[beta], interleukin-6 and interleukin-8 messenger ribonucleic acid (mRNA) in the lower uterine segment during term parturition. Study design: mRNA extracts from the lower uterine segment obtained from 53 women undergoing non-elective caesarean section at term were analyzed by semi-quantitative reverse transcriptase polymerase chain reaction. The patients were grouped according to cervical dilatation (less than 2 cm, n=18; 2 to less than 4 cm, n=13; 4-6 cm, n=7; more than 6 cm, n=15) at the time of caesarean section. Results: Interleukin-1[beta] and interleukin-8 mRNA-contents at more than 6 cm cervical dilation were significantly higher than at less than 2 cm. The levels of interleukin-6 were already significantly increased in the 4-6 cm group, while the tumor necrosis factor [alpha] ribonucleic acid content did not change during parturition. Conclusion: The process of cervical dilatation during parturition at term is associated with an increased expression of interleukin-1[beta], interleukin-6 and interleukin-8 mRNA in the lower uterine segment. These findings support the theory that the activation of the inflammatory network plays an important role in the progress of cervical dilatation.

European Journal of Paediatric Neurology 1


http://www.sciencedirect.com/science/article/B6WF2-4DJBP8R-2/2/ea4a5ce8e6bcbfe42eca8f6b7bfa6b

SummaryNADH-ubiquinone oxidoreductase or complex I deficiency is a frequently diagnosed enzyme defect of the oxidative phosphorylation (OXPHOS) system in humans. However, in many patients, with complex I deficiency and clinical symptoms suggestive of mitochondrial disease, often no genetic defect can be found after investigation of the most common mitochondrial DNA (mtDNA) mutations. In this study, 20 patients were selected with a biochemically documented complex I defect and no common mtDNA mutation. We used the Denaturing Gradient Gel Electrophoresis (DGGE) method with primers encompassing all mitochondrial encoded fragments, to search in a systematic manner for mutations in the mitochondrial genome of complex I. In our group of patients, we were able to detect a total of 96 nucleotide changes. We were not able to find any disease causing mutation in the mitochondrial encoded subunits of complex I. These results suggested that the complex I deficiency in this group of patients is most probably caused by a defect in one of the nuclear encoded structural genes of complex I, or in one of the genes involved in proper assembly of the enzyme.

European Journal of Pharmaceutical Sciences 3
PCR amplification has emerged as a very important tool in biological research. The utility of the PCR is, however, hampered by the fact that it is a slow technique. Faster heating cycles are therefore needed, both to enhance the activity of the enzyme, and to enable shortening of the reaction times. In this paper, polymerase chain reactions with focused microwave irradiation as the source of heat were demonstrated for the first time. Thus, it was established that continuous microwave heating does not terminate the enzymatic function of the polymerase. The results indicate the possibility to shorten the total reaction time. In addition, the technique may give the possibility to perform PCR reactions in millilitre scale.

The effects of 12 Ca2+ antagonists on MDR1 were examined by two independent models: the inhibitory effect on MDR1-mediated transport of [3H]digoxin using MDR1-overexpressing LLC-GA5-COL150 cell monolayers and the reversal effect on cytotoxicity of vinblastine or paclitaxel using MDR1-overexpressing Hvr100-6 cells. The inhibitory effects on [3H]digoxin transport were assessed as the 50% inhibitory concentration during 4 h exposure, and the values were the lowest for nicardipine (4.54 [μM]), manidipine (4.65 [μM]) and benidipine (4.96 [μM]), followed by bepridil (10.6 [μM]), barnidipine (12.6 [μM]), efondipine (13.0 [μM]), verapamil (13.2 [μM]) and nilvadipine (18.0 [μM]). The reversal effect on cytotoxicity was assessed by the 50% growth inhibitory concentration after 3 days exposure, and the resistance to vinblastine or paclitaxel in Hvr100-6 cells was reversed by manidipine, verapamil, benidipine, barnidipine, and nicardipine, in that order. Bepridil, barnidipine, efondipine, verapamil and nilvadipine showed similar inhibitory effects on [3H]digoxin transport, but barnidipine and verapamil showed a stronger effect in reversal of cytotoxicity. Real-time quantitative RT-PCR assay indicated a decrease in MDR1 mRNA expression by barnidipine and verapamil. It is concluded that Ca2+ antagonists cannot only be direct inhibitors of MDR1 but that some may act as inhibitors of expression of MDR1 via down-regulation of MDR1 mRNA.

The effect of electric field gradients are examined on the speed, selectivity, read length, and accuracy for DNA sequencing using capillary array electrophoresis. Modified electric field gradients was realized to read over 800 bases within 140 min. The method developed is
effectively applicable to single nucleotide polymorphism analysis for genomic drug discovery and pharmacogenomics.

**European Journal of Pharmacology** (30)


http://www.sciencedirect.com/science/article/B6T1J-3S0MJNV-J/2/8f2479761514a91b02e87b89f1872d3e

Modifications of rat prostatic [\(\alpha\)]1-adrenoceptors were investigated in testosterone-induced prostatic hypertrophy. prazosin bound to a single class of binding sites with a dissociation constant of 57.9 +/- 5.02 pM. The greater part of the binding capacity (24.6 +/- 1.02 fmol/mg protein) was made up of chloroethylclonidine-resistant binding sites that showed high-affinity for oxymetazoline and 5-methyl-urapidil, and was identified as [\(\alpha\)]1A-adrenoceptors. The remaining chloroethylclonidine-sensitive binding sites that showed low-affinity for oxymetazoline and 5-methyl-urapidil were preferentially identified as [\(\alpha\)]1B-adrenoceptors. mRNA for the three [\(\alpha\)]1-adrenoceptors ([\(\alpha\)]1a, [\(\alpha\)]1b and [\(\alpha\)]1d) was detected. Testosterone administration produced a 23% decrease of [\(\alpha\)]1-adrenoceptor density, likely by an increase of prostatic glandular epithelium and a decrease in the relative proportion of smooth muscle, thus of [\(\alpha\)]1-adrenoceptor density. The steady state level of mRNAs for [\(\alpha\)]1-adrenoceptors was not modified by testosterone treatment. These results indicate that prostate [\(\alpha\)]1-adrenoceptors are not affected in the prostatic hypertrophy induced by testosterone.


http://www.sciencedirect.com/science/article/B6T1J-3W2Y75F-1J/2/8590278bef2b76e9135e13f4e547e727

To elucidate which neuropeptide Y receptor subtype is responsible for the neuropeptide Y-induced potentiation of the noradrenaline-evoked contraction in human omental arteries we used antisense oligodeoxynucleotide (Antisense), the new selective neuropeptide Y Y1 receptor antagonist, BIBP3226 ((R)-N2-(diphenylacetyl)-N-[(4-hydroxyphenyl) methyl]--arginine-amide) and the reverse transcriptase-polymerase chain reaction (RT-PCR). Neuropeptide Y significantly potentiated the noradrenaline-induced contraction in non-incubated vessels (pEC50 6.4 +/- 0.2 vs. 5.9 +/- 0.2) and in vessels incubated with 1 [\(\mu\)]M Sense oligodeoxynucleotide (Sense) (pEC50 6.0 +/- 0.1 vs. 5.6 +/- 0.2). In vessels incubated with 1 [\(\mu\)]M Antisense the potentiating effect of neuropeptide Y was completely ablished. BIBP3226 (1 [\(\mu\)]M) inhibited the neuropeptide Y-induced potentiation in human omental arteries (pEC50 5.8 +/- 0.3 vs. 6.4 +/- 0.2). Finally, messenger RNA for the neuropeptide Y Y1 receptor was detected using RT-PCR. On the basis of our results we conclude that the neuropeptide Y-induced potentiation of the noradrenaline-induced contraction is mediated by the neuropeptide Y Y1 receptor.

http://www.sciencedirect.com/science/article/B6T1J-44VGBP2-2/2/73024e5e161a11c45e9c1721813a05d

Using a combination of reverse transcription polymerase chain reaction (RT-PCR) and inverse-PCR techniques, we amplified, cloned and sequenced a full-length porcine 5-hydroxytryptamine 1F (5-ht1F) receptor complementary DNA (cDNA) derived from porcine trigeminal ganglion. Sequence analysis revealed 1101 base pairs (bp) encoding an open reading frame of 366 amino acids showing a high similarity (>90%) with the 5-ht1F receptor sequences from other species, including human. The recombinant porcine 5-ht1F receptor was expressed in African green monkey kidney cell lines (COS-7 cells) and its ligand binding profile was determined using [3H]5-HT. The affinities of several agonists (LY334370 (5-(4-fluorobenzoyl)amino-3-(1-methylpiperidin-4-yl)-1H-indole fumarate)>CP122638 (N-methyl-3 [pyrrolidin 2(R)-yl methyl]-1H-indol-5-ylmethyl sulphonamide)=naratriptan=5-HT>eletriptan>sumatriptan=frovatriptan=avitriptan=dihydroergotamine>zolmitriptan=5-carboxamidotryptamine=rizatriptan>alniditan=donitriptan=L694247 (2-[5-[3-(4-methylsulphonylamino)benzyl]-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl) ethylamine) and putative antagonists (methiothepin>GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl 4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride)>ritanserin>SB224289 (2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo [2,3-f] indole-3-spiro-4'-piperidine hydrochloride)>BRL155572 ([1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropyl)piperazin] hydrochloride)>ketanserin=pindolol) correlated highly with those described for the recombinant human 5-ht1F receptor (Spearman correlation coefficient; rs=0.942). Nevertheless, as compared to the human homologue, some triptans (i.e. sumatriptan, zolmitriptan and rizatriptan) displayed a 10- to 15-fold lower affinity for the porcine 5-ht1F receptor. Using RT-PCR technique, the expression of porcine 5-ht1F receptor mRNA was observed in cerebral cortex, trigeminal ganglion and several blood vessels, but not in skeletal muscles. In conclusion, we have cloned and established the amino acid sequence and ligand binding profile of the porcine 5-ht1F receptor as well as the distribution of its mRNA. This information may be helpful in exploring the role of 5-ht1F receptor in physiological processes and diseases, such as migraine.


http://www.sciencedirect.com/science/article/B6T1J-3W0NCT2-9/2/81d585cd506a8dc96060f1bb62fd59e4

To investigate the effect of cyclooxygenase inhibition in experimental Gram-negative sepsis, indomethacin was administered to mice at different times (1 or 5 days, or 1 h) before sublethal infection with an intravenous inoculum of Pseudomonas aeruginosa. Early indomethacin exposure did not alter the outcome of infection, yet treatment at the time of bacterial challenge resulted in a high mortality rate. Polymerase chain reaction-assisted mRNA amplification in the spleens of infected mice revealed that tumor necrosis factor [alpha] (TNF-[alpha]) messenger was selectively expressed by the drug-treated and infected mice during the 24 h preceding death. Higher TNF-[alpha] levels were found in sera from these mice, whose macrophages produced increased levels of nitric oxide in vitro. Both pentoxifylline, an inhibitor of TNF-[alpha] synthesis, and an inhibitor of nitric oxide production improved survival in the indomethacin-treated and infected mice, although no such effect followed the administration of TNF-neutralizing antibodies. These data support the notion that cyclooxygenase inhibitors may exert both positive and
negative effects in Gram-negative sepsis, the latter presumably involving overproduction of TNF- [alpha].


http://www.sciencedirect.com/science/article/B6T1J-4967NBX-5/2/547e12a57790dba51de69c846eeea901

Thromboxane A2 has been implicated as a mediator of bronchial hyperresponsiveness in asthma. Modulating agents are currently marketed in Japan and under clinical evaluation in the US, but full characterization of the thromboxane A2 receptor and the signaling pathways that link it to the proliferative events taking place during airways structural remodeling has not been achieved. Here, we report that the presence of mRNA for both [alpha] and [beta] isoforms of the thromboxane A2 receptor in smooth muscle cells from human bronchi correlates with protein expression evaluated by radioligand binding of the antagonist, SQ29,548 ([1S-[1[alpha],2[alpha](Z),3[alpha],4[alpha]]-7-[3-[[2-[[phenylamino]carbonyl]hydrazino)methyl]7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic-acid) (Kd=3.4 nM+/−44%CV, coefficient of variation, Bmax=41 fmol/mg prot+/−38%CV). The receptor is functional, as the agonist, U46619 (9,11-dideoxy-9[alpha],11[alpha]-methanoepoxy-prosta-5Z,13E-dien-1-oic-acid), induced a concentration-dependent Ca2+ transient (EC50=0.12 [mu]M+/−27%CV). Furthermore, U46619 concentration dependently increased DNA synthesis and markedly potentiated the epidermal growth factor mitogenic effect. Both events were specifically inhibited by SQ29,548, independently from transactivation of the epidermal growth factor receptor and partially sensitive to pertussis toxin.


http://www.sciencedirect.com/science/article/B6T1J-400WXW3-D/2/34558dcb4dcd7821c338b134c7fb97f0c

Recombinant fractalkine possesses both chemoattractive and adhesive properties in vitro. Previous studies have demonstrated an upregulation of this molecule on the membranes of activated human endothelial cells and hypothesised that fractalkine plays a role in the recruitment and adherence of monocytes to the activated endothelium. Here we present data analysing both the adhesive and chemoattractive properties of this chemokine expressed by activated human umbilical vein endothelial cells. We demonstrate that both recombinant fractalkine and endogenously produced fractalkine function as adhesion molecules, tethering monocytes to the endothelium. However, our data demonstrate that although recombinant fractalkine has the potential to function as a potent monocyte chemoattractant, the endogenous fractalkine cleaved from activated human umbilical vein endothelial cells is not responsible for the observed chemotaxis in this model. Instead, we show that monocyte chemoattractant protein-1 (MCP-1), secreted from the activated human umbilical vein endothelial cells, is responsible for the chemotaxis of these monocytes.

Glucocorticoid-induced apoptosis is a well-recognized physiological regulator of T-cell number and function. Alisol B acetate, a triterpene from Alisma Plantago-aquatica, has a glucocorticoid-like structure, and may have a similar function like glucocorticoid-induced apoptosis in both vascular smooth muscle cell line (A7r5) and human acute lymphoblastic leukemia cell line (CEM cells). For exploring its mechanism, mitochondria membrane potential and apoptosis-related gene expression were discussed. Alisol B (10-6-10-4 M) inhibited serum-stimulated DNA synthesis in a concentration-dependent manner (IC50=4.0+/-0.8 x 10-6 M in A7r5 and 2.1+/-1.2 x 10-6 M in CEM cells). The cell viability was reduced at 10-4 M of alisol B. Similar results were seen in dexamethasone treatment (a synthetic glucocorticoid, 10-6 M, 48 h). Apoptosis was induced after the cells were exposed to 10-5-10-4 M alisol B or 10-6 M dexamethasone for 48 h. The mitochondrial membrane potential (ΔΨm) was significantly reduced after the alisol B treatment, indicating that the mitochondria might play a role in the alisol B induced cell apoptosis. Alisol B (10-5-10-4 M) increased the levels of c-myc and bax mRNA and proteins, but not on the anti-apoptotic proto-oncogene, bcl-2, in A7r5 and CEM cells. In contrast, dexamethasone (10-6 M) treatment only caused significant increase in c-myc mRNA levels. These results suggest that the increased ratio of Bax/Bcl-2 and the decreased mitochondrial membrane potential might be involved in the mechanisms of alisol B-induced cell apoptosis.


activity and protein were higher in the fibroblasts and smooth muscle cells than in endothelial cells. Neutral endopeptidase inhibition prevented atrial natriuretic peptide (ANP) degradation in endothelial and smooth muscle cells. It potentiated ANP-stimulated cyclic GMP production in these cells. Neutral endopeptidase inhibition also reduced bradykinin degradation and potentiated bradykinin-stimulated release of arachidonic acid in fibroblasts and endothelial cells. Our data demonstrate the presence and functional activity of neutral endopeptidase in all three cell layers of rat aorta as well as in primary cells of the vessel. The data suggest that local concentrations of vasoactive peptides in the vessel wall might be regulated by the neutral endopeptidase cleavage pathway in the immediate vicinity of their target cells.


http://www.sciencedirect.com/science/article/B6T1J-46NYFT4-3/2/419756788c5c946fbdbbda20c550d7d0

TAK-778 [(2R,4S)-(−)-N-(4-Diethoxyphosphorylmethylphenyl)-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepin-2-carboxamide: mw 505.52], a novel compound promoting osteoblast differentiation, promotes osteogenesis in vitro and enhances bone formation during skeletal repair in vivo. In this study, we further evaluated the effects of TAK-778 on the differentiation of cultured bone marrow stromal cells into osteoblasts in the presence of dexamethasone, paying particular attention to the expression of transcription factors involved in regulating osteoblast differentiation. Treatment of TAK-778 (10−7-10−5 M) for 4 h resulted in an increase in the mRNA expression of Msx2, but not Cbfa1 or Dlx5. This transcriptional alteration preceded the changes in other markers related to the osteoblast phenotype, such as alkaline phosphatase and osteocalcin mRNA. The transfection of Msx2-antisense in the cells caused a significant reduction in the levels of alkaline phosphatase mRNA expression induced by TAK-778. These results suggest that TAK-778 promotes osteoblast differentiation partly through the expression of Msx2, a homeobox-related gene.


http://www.sciencedirect.com/science/article/B6T1J-3RSGTV0-X/2/bbd6fc996305673934bf45693cfd3d92

Nitric oxide (NO) synthesis may be coupled to the activity of the cellular -arginine transporter, namely the cationic amino acid transporter. The present study examined tumor necrosis factor (TNF)-[alpha]-induced alterations in the gene expression of the cationic amino acid transporter (CAT) and NO production in human umbilical vein endothelial cells. In quiescent endothelial cells, CAT-1 mRNA expression, determined by reverse transcription-polymerase chain reaction, was dominant to that of CAT-2. TNF-[alpha] (10 ng/ml for 1-24 h) induced a time-dependent increase in CAT-2 but not CAT-1 expression. Moreover, TNF-[alpha] (1-30 ng/ml) treatment for 6 h induced a concentration-dependent increase in CAT-2 mRNA expression. The upregulation of CAT-2 expression by TNF-[alpha] was associated with enhanced nitrite accumulation in the culture medium (70% increase compared with vehicle-treated cells at 24 h). Thus, induction of the cationic amino acid transporter may constitute one mechanism for the TNF-[alpha]-induced NO production in human umbilical vein endothelial cells.

http://www.sciencedirect.com/science/article/B6T1J-3W1YHDB-G/2/f34e18193a851079e39fa216f816dd2f

Vascular cell adhesion molecule-1 (VCAM-1) is a mononuclear leukocyte-selective adhesion molecule that is expressed in human vascular endothelial cells at sites of local inflammation. It participates in local endothelial-monocyte interactions during the initiation of atherosclerosis. In the present study, endothelin alone did not induce the surface expression and mRNA accumulation of VCAM-1 in human vascular endothelial cells, but inhibition of endogenous nitric oxide (NO) by NG-monomethyl--arginine enhanced the surface expression and mRNA accumulation of VCAM-1 stimulated by endothelin-1. It is conceivable that in human vascular endothelial cells, stimulation of an endothelin receptor results in the production of nitric oxide (NO), suppressing the expression of VCAM-1. Endothelin-1 enhanced the surface expression and mRNA accumulation of VCAM-1 in cells treated with tumor necrosis factor [alpha] (TNF-[alpha]). The enhancement by endothelin-1 may be explained by the inhibitory effect of TNF-[alpha] on endothelin-induced NO production. Pretreatment with BQ788 (an endothelin ETB receptor antagonist) or inhibitors of nuclear factor kappa B (NF-[kappa]B) activation completely diminished the synergistic enhancement of VCAM-1 expression by endothelin-1 in TNF-[alpha]-stimulated vascular endothelial cells, both at the protein and mRNA levels. These findings suggest that the synergistic enhancement of VCAM-1 expression by TNF-[alpha] and endothelin ETB receptor stimulation may be augmented by the induction of NF-[kappa]B binding activity in human vascular endothelial cells.


http://www.sciencedirect.com/science/article/B6T1J-4DDRBGW-4/2/3f37c4156f40a035e04a49aa87e6d27d

Central glucocorticoid receptor function may be reduced in depression. In vivo modelling of glucocorticoid receptor underfunctionality would assist in understanding its role in depressive illness. The role of glucocorticoid receptors in modulating 5-HT2A receptor expression and function in the central nervous system (CNS) is presently unclear, but 5-HT2A receptor function also appears altered in depression. With the aid of RNAse H accessibility mapping, we have developed a 21-mer antisense oligodeoxynucleotide (5'-TAAAAACAGGCTTCTGATCCT-3', termed GRAS-5) that showed 56% reduction in glucocorticoid receptor mRNA and 80% down-regulation in glucocorticoid receptor protein in rat C6 glioma cells. Sustained delivery to rat cerebral ventricles in slow release biodegradable polymer microspheres produced a marked decrease in glucocorticoid receptor mRNA and protein in hypothalamus (by 39% and 80%, respectively) and frontal cortex (by 26% and 67%, respectively) 5 days after a single injection, with parallel significant up-regulation of 5-HT2A receptor mRNA expression (13%) and binding (21%) in frontal cortex. 5-HT2A receptor function, determined by DOI-head-shakes, showed a 55% increase. These findings suggest that central 5-HT2A receptors are, directly or indirectly, under tonic inhibitory control by glucocorticoid receptor.

Soluble guanylyl cyclase activity and its stimulation by diethylamine NONOate was measured in aortae from hypertensive TGR (mREN2)27 rats (TGR) and Sprague-Dawley controls. Superoxide dismutase was added in vitro to evaluate the contribution of oxidative breakdown of nitric oxide (NO) by superoxide anions. Expression of soluble guanylyl cyclase was assessed by reverse transcriptase-polymerase chain reaction (RT-PCR). Basal and stimulated soluble guanylyl cyclase activity was significantly reduced in TGR rats, addition of superoxide dismutase had no effect. Expression of soluble guanylyl cyclase subunits was not different between strains. The independent contribution of hypertension and the overactive renin-angiotensin system to soluble guanylyl cyclase subsensitivity was assessed after normalization of TGR's blood pressure by the Ca2+-channel blocker amlodipine or the angiotensin converting enzyme-inhibitor enalapril. Soluble guanylyl cyclase activity in TGR was slightly increased by amlodipine and almost completely restored by enalapril. In conclusion, TGR showed desensitized vascular soluble guanylyl cyclase, depending on their overactive renin-angiotensin system.


Inflammatory gene expression in airway smooth muscle may be influenced by its inflammatory milieu. We analysed the gene expression profile of airway smooth muscle cells cultured from human airways exposed to a pro-inflammatory cytokine, interleukin-1[beta], a T helper-2 cytokine, interleukin-13, and to a growth factor, transforming growth factor (TGF)[beta]1 (10 ng/ml each) after 4 and 24 h using the Affymetrix GeneChip 95A array which detects 12,500 genes and expression sequence tags (ESTs). Airway smooth muscle cells were responsive to each cytokine with distinctive patterns of gene expression for cytokines, chemokines, adhesion and signalling proteins, and transcription factors. Interleukin-1[beta] induced the highest number of genes such as cytokines/chemokines including interleukin-8, growth-related oncogene (GRO)-[alpha], -[beta] and -[gamma], epithelial neutrophil activating protein (ENA)-78, monocyte chemotactic protein (MCP)-1, -2 and -3 and eotaxin. Using quantitative real-time reverse transcription-polymerase chain reaction, the expression of GRO-[alpha], -[beta] and -[gamma], interleukin-8 and eotaxin by interleukin-1[beta] was confirmed, with good correlation with microarray data. Transforming growth factor (TGF)[beta]1 induced other growth factors such as connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and many structural and extracellular matrix proteins. Interleukin-13 was the weakest inducer, with stimulation of eotaxin and genes of unknown function. While many genes were co-regulated at 4 and 24 h, there were also differences in expression patterns. Interleukin-1[beta] induces a predominantly pro-inflammatory profile while TGF[beta]1 can be linked to proliferative and matrix changes. The rich profile of mediators, growth factors and signalling molecules released from airway smooth muscle depends on the inflammatory milieu.

The metabolic response of galanin GAL1 receptor subtype, endogenously expressed in human Bowes melanoma (HBM) cells, was investigated. Cytosensor microphysiometry was used to determine the extracellular acidification rate. A biphasic response, consisting of a rapid increase in the extracellular acidification rate followed by a decrease below the basal level, was observed after perfusion with human galanin. The magnitude and the rate of onset of both phases were dependent on the galanin concentration. The increase in the extracellular acidification rate (maximum of 25% of basal level; -log(EC50)=7.23 +/- 0.14) was transient, whereas the following decrease (maximum of 40% of basal level; -log(EC50)=7.77 +/- 0.23) was sustained. The EC50 values for the increase and decrease were in a similar range. After consecutive galanin administration, the magnitude of the response was the same as for the unexposed cells, indicating the absence of galanin receptor desensitization or internalization in HBM cells. Responses were blocked by pretreatment with pertussis toxin and phorbol-12-myristate-13-acetate (PMA), indicating a G-protein/protein kinase C signalling pathway. Our microphysiometry results show a biphasic response of the extracellular acidification rate mediated by the galanin receptor expressed in HBM cells which has not been described previously for any other endogenously expressed neuropeptide receptor.


http://www.sciencedirect.com/science/article/B6T1J-4B233CJ-2/2/cfcd387690503226eb8ef8c34cac71a4

The aim of this study was to characterise the expression of the melanocortin system in the normal and injured rat visual system. Using real-time polymerase chain reaction and immunohistochemistry, we detected melanocortin MC3, MC4 and MC5 receptors and proopiomelanocortin in adult retina and superior colliculus. Melanocortin MC4 receptor mRNA was the most abundant receptor. Melanocortin MC3, MC4 and MC5 receptors were localised to the ganglion cell and inner nuclear layers and the melanocortin MC3 and MC4 receptors were localised to retinal ganglion cells. Transection of the optic nerve leads to ganglion cell death and both melanocortin receptor and proopiomelanocortin expression decreased in superior colliculus after transection whereas the expression was unchanged or even increased in the retina. [alpha]-Melanocyte-stimulating hormone elicited neurite outgrowth from embryonic retinal explants. Together, these data implicate a role for the melanocortin system in the adult rat retina and that melanocortins can stimulate neurite growth from retinal neurons.


http://www.sciencedirect.com/science/article/B6T1J-47DTGYC-1/2/9c6ba3a0538c559e26c0b1311ad0ccff

Extracellular nucleotides were used to characterise the contractile P2 receptors in the rat basilar artery. The isometric tension was recorded in vitro and receptor mRNA expression was examined by reverse transcriptase polymerase chain reaction (RT-PCR) after endothelium-denudation. Transient vasoconstriction was evoked by [alpha][beta]-methylene-adenosine triphosphate ([alpha][beta]-MeATP), indicating the presence of P2X1 receptors. The P2Y receptors were analysed after P2X receptor desensitisation with 10 [mu]M [alpha][beta]-MeATP. Uridine diphosphate (UDP) and uridine triphosphate (UTP) induced sustained contractions of similar magnitude. The stable nucleotide analogue, uridine 5'-O-thiodiphosphate (UDP[beta]S), was
clearly more potent than uridine 5'-O-3-thiotriphosphate (UTP[gamma]S), suggesting prominent contractile effects of P2Y6 receptors. P2Y2 and P2Y4 receptors might also be involved in nucleotide responses, since UTP[gamma]S and adenosine 5'-O-3-thiotriphosphate (ATP[gamma]S) were of similar potency. The P2Y1 selective agonists, adenosine 5'-thiodiphosphate (ADP[beta]S) and 2-methylthioadenosine diphosphate (2-MeSADP) did not induce contractions. RT-PCR analysis demonstrated P2X1, P2Y1, P2Y2 and P2Y6 receptor mRNA expression, while the P2Y4 band was weak. In conclusion, extracellular nucleotides induce contractions of cerebral arteries primarily by activation of P2Y6 receptors on smooth muscle cells, with a lesser contribution of P2Y2 and P2X1 receptors. Although mRNA for the P2Y1 receptor was detected by RT-PCR, it does not mediate contraction.


http://www.sciencedirect.com/science/article/B6T1J-47XWTYY-5/2/0a299d48ee1555fa90fe9af94f3e38b7

Adrenomedullin is a hypotensive peptide secreted from various cells. Recently, we found that adrenomedullin, but not calcitonin gene-related peptide (CGRP), stimulates histamine release from rat peritoneal mast cells. In the present studies, we investigated the expression of mRNA for calcitonin-receptor-like receptor (CRLR) and receptor-activity modifying proteins (RAMPs), the components of proposed adrenomedullin receptors, in rat peritoneal mast cells by reverse transcription-polymerase chain reaction (RT-PCR). Results revealed that mRNA for CRLR, RAMP2 and RAMP3 was expressed in rat peritoneal mast cells, whereas mRNA for RAMP1 was not. These data suggest that adrenomedullin might stimulate histamine release via its proposed receptor (CRLR/RAMP2 or 3), rather than via the CGRP receptor (CRLR/RAMP1).


Guanosine 3',5'-cyclic monophosphate (cGMP) has an important role in regulating vascular smooth muscle tone. We examined whether mRNA for multidrug resistance protein (MRP) 4 and MRP5, which were recently identified as ATP-dependent export pumps for cyclic nucleotides, is expressed in the porcine coronary and pulmonary arteries. The results showed that both arteries express mRNA for MRP4 and MRP5, and thus these proteins may be novel targets for the prevention and/or treatment of various cardiovascular diseases.


http://www.sciencedirect.com/science/article/B6T1J-40N7DXF-3/2/6b0442c7eb212f0ecce8d4e81a3555d0

Ferrets (Mustela putorius furo) are useful animals for determining anti-emetic activity via 5-HT3 receptors in vivo. We isolated a cDNA encoding the 5-hydroxytryptamine (5-HT) type 3A receptor
subunit (5-HT3A) from ferret colon, expressed it in a human embryonic kidney cell line and determined its pharmacological properties. The open reading frame of the isolated cDNA encoded a 483-amino acid protein, corresponding to the shorter splice variant of 5-HT3A receptors. Splice variants were no longer detected by reverse transcriptase-polymerase chain reaction. The ferret 5-HT3A receptor exhibits a high degree of amino acid sequence identity (>=80%) to that of other species. Binding studies demonstrated the following rank order of potency for agonists: meta-chlorophenylbiguanide (mCPBG)>2-methyl-5-hydroxytryptamine (2-Me-5-HT)=5-HT, and for antagonists: ondansetron=tropisetron>(+)-tubocurarine>metoclopramide. Electrophysiological studies revealed that mCPBG was a partial agonist and 2-Me-5-HT was an almost fully effective agonist compared to 5-HT.

http://www.sciencedirect.com/science/article/B6T1J-3S3M07V-Y/2/7db2a6094c339dc5b2e3f82602e28dd6

The aim of the present study was to investigate the level of regulation of the contractile endothelin ETB receptor which appears spontaneously after organ culture of vascular segments. Endothelin-1 elicited a strong contraction while the selective endothelin ETB receptor agonist, sarafotoxin 6c, had a negligible effect on fresh ring segments of rat mesenteric artery. After organ culture in serum-free Dulbecco's modified Eagle's medium at 37[deg]C (for 1 or 2 days) the endothelin-1-induced contraction was unchanged, whereas sarafotoxin 6c induced, after 1 day, a marked contraction which was further increased at day 2. The contraction induced by sarafotoxin 6c was significantly attenuated by the transcriptional inhibitor, actinomycin D, or the translational inhibitor, cyclohexamide, while the endothelin-1-induced contraction was much less affected. mRNA for endothelin ETA and endothelin ETB receptors was present in fresh human omental arteries denuded of endothelium. However, after organ culture, endothelin ETB mRNA was more prominent than endothelin ETA mRNA. Furthermore, the mRNA for both receptors was decreased after treatment with actinomycin D but not with cyclohexamide. This suggests that the endothelin ETA receptor is the dominating contractile receptor in fresh arteries while organ culture induces transcription and subsequent translation of contractile endothelin ETB receptors.

http://www.sciencedirect.com/science/article/B6T1J-4D5PB5H-6/2/8197e9c6621e7032630d6b508debc8fc

Besides possessing a strong growth hormone (GH)-releasing activity, the gastrointestinal octanoylated peptide ghrelin has been reported to antagonize lipolysis in rat adipocytes. It is not yet clear whether this inhibitory activity on lipolysis is also shared by the major circulating isoform, des-acyl ghrelin, that does not activate the ghrelin receptor, namely the type 1a GH secretagogue-receptor (GHS-R1a) and lacks the endocrine effects of the acylated form. Here we show that des-acyl ghrelin, like ghrelin and some synthetic GHS (hexarelin and MK0677) and carboxy-terminally ghrelin fragments such as ghrelin-(1-5) and ghrelin-(1-10), all significantly reduced, over concentrations ranging from 1 to 1000 nM, the stimulation of glycerol release caused in rat epididymal adipocytes by the nonselective [beta]-adrenoceptor agonist isoproterenol in vitro. The order of potency on stimulated-lipolysis was: des-acyl ghrelin=ghrelin>MK0677=hexarelin>ghrelin-(1-5)=ghrelin-(1-10). This ranking was consistent with the binding experiments performed on membranes of epididymal adipose tissue or isolated
adipocytes that did not express mRNA for GHS-R1a. A common high-affinity binding site was recognized in these cells by both acylated and des-acylated ghrelin and also by hexarelin, MK0677, ghrelin-(1-5) and ghrelin-(1-10). In conclusion, these findings provide the first evidence that des-acyl ghrelin, as well as ghrelin, short ghrelin fragments and synthetic GHS, may act directly as antilipolytic factors on the adipose tissue through binding to a specific receptor which is distinct from GHS-R1a.


http://www.sciencedirect.com/science/article/B6T1J-3S3N78D-1F/2/744ca226ce6d072a07018fed28489c20

Using the polymerase chain reaction with degenerate primers to identify novel G-protein-coupled receptors of the rat alveolar Type II cell, we identified sequences expressed by the Type II cell identical to the sequence of the rat brain cannabinoid receptor (CB1). The use of Northern blot analysis to examine expression of CB1 mRNA in rat tissues revealed differences between the brain and lung. While rat brain expressed a 6.0 kb mRNA as previously described, rat lung expressed mRNA of 4.5 and 6.0 kb. Isolated lung alveolar Type II cells also expressed mRNA of 4.5 and 6.0 kb as determined by Northern analysis. However, only freshly isolated Type II cells contained cannabinoid receptor mRNA. Reverse transcriptase-polymerase chain reaction (RT-PCR) failed to detect CB1 mRNA in Type II cells maintained in culture for 1 or 2 days. We next determined developmental changes in lung CB1 mRNA expression using semi-quantitative RT-PCR. CB1 expression was detected as early as gestational day 16 in rat lung and mRNA levels increased to fetal day 20 before birth, before declining to adult levels. Fetal rat lung explants were utilized to further examine the ontogeny and hormonal effects on CB1 mRNA expression. Hydrocortisone induced a dose-dependent expression in 15-day and 18-day explants, similar to previous results for surfactant-associated proteins. Our results demonstrate expression of CB1 mRNA in rat alveolar Type II cells and rat lung. This expression is ontogenically and hormonally regulated, with maximal expression noted just prior to birth in rat lung. Since CB1 mRNA is only expressed in freshly isolated Type II cells, CB1 may be useful as a Type II cell marker.


http://www.sciencedirect.com/science/article/B6T1J-44NM99X-5/2/c4fc201e561f61b72aa261b0269

Properties of inwardly rectifying K+ channels in small-cell lung cancer (SCLC) cells have not been clarified in detail. Here, we found inwardly rectifying K+ channels in a human SCLC cell line (RERF-LC-MA), which expresses no multidrug resistance-associated protein 1 (MRP1) and multidrug resistance P-glycoprotein (MDR1). Extracellular Ba2+ and Cs+ inhibited inwardly rectifying K+ currents of RERF-LC-MA cells in a concentration-dependent manner, but tetraethylammonium ion and glibenclamide were ineffective. Okadaic acid, an inhibitor of phosphatases 1 and 2A, and phorbol-12,13-dibutyrate, an activator of protein kinase C, significantly decreased the inwardly rectifying K+ current. Lowering the intracellular pH but not the extracellular pH decreased the K+ current. Reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting analysis showed that RERF-LC-MA cells express Kir2.1 mRNA and protein. The inwardly rectifying K+ current is suggested to be generated by Kir2.1 protein in the human small-cell lung cancer cell, and that the K+ channel is negatively regulated by protein kinase C and the intracellular acidic pH.
To examine the effects of lansoprazole, a proton pump inhibitor, on rhinovirus infection in airways, human tracheal epithelial cells were infected with a major subgroup of rhinoviruses, type 14 rhinovirus. Rhinovirus increased the mRNA expression of intercellular adhesion molecule-1 (ICAM-1) in the cells, the major rhinovirus receptor, and the content of the soluble form of ICAM-1 (sICAM-1) and cytokines in supernatants. Lansoprazole reduced supernatant titers and RNA of rhinovirus, the susceptibility to rhinovirus infection, the ICAM-1 mRNA production, the number and fluorescence intensity of acidic endosomes in the cells, and supernatants sICAM-1 and cytokine concentrations including interleukin-$\beta$. Antibody to interleukin-$\beta$ reduced baseline and rhinovirus-induced ICAM-1 production. These results suggest that lansoprazole inhibits rhinovirus infection by reducing ICAM-1 via partly endogenous production of interleukin-$\beta$, and by blocking the rhinovirus RNA entry into the endosomes. Lansoprazole may modulate airway inflammation by reducing the production of cytokines and ICAM-1 in rhinovirus infection.

The affinity, selectivity and agonistic properties of a constrained dopaminergic compound, the benz[e]indole cis-8-hydroxy-3-(n-propyl)1,2,3a,4,5,9b-hexahydro-1H-benz[e]indole (cis-8-OH-PBZI), for the dopamine D3 receptor were evaluated in competition binding experiments with cloned human dopamine receptor subtypes and, to further extend its profile, in vitro radioligand binding assays. The $K_i$ value measured for competition binding of this compound to the dopamine D3 receptor was 27.4+/-3.1 nM; this was 775-fold, 550-fold, 90-fold and 10-fold higher affinity than that measured at dopamine D1A, D5, D2s and D4 receptors, respectively. Evidence of dopamine receptor activation by cis-8-OH-PBZI was obtained by measuring dose-dependent increases in extracellular acidification rates and decreases in cAMP synthesis. In vivo, cis-8-OH-PBZI potently induced Fos protein immunoreactivity in the rat medial prefrontal cortex and shell region of the nucleus accumbens, but only marginally in the motor dorsolateral striatum, indicating a selective limbic site of action. In conclusion, the present data identify cis-8-OH-PBZI as having preference for the dopamine D3 receptor in vitro, and as having dopamine agonist activity and limbic sites of action in vivo.

The molecular properties of the sulfonylurea receptor 2 (SUR2) subunits of KATP channels
expressed in urinary bladder were assessed by polymerase chain reaction (PCR). This showed that SUR2B exon 17- mRNA (72%) was predominant over the SUR2B exon 17+ splice variant (28%). The pharmacological properties of both of these isoforms stably expressed in mouse Ltk-cells (L-cells) with KIR 6.2 were determined by measuring changes in membrane potential responses evoked by K+ channel openers using bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)) fluorescence. The rank order potency of a variety of structurally distinct K+ channel openers was found to be the same in both stable cell lines and compared well with guinea pig bladder cells. The potency of these compounds in the SUR2B exon 17- cells more closely resembled the potency measured in guinea pig bladder unlike the cell line containing the SUR2B exon 17+ subtype. Analysis of the displacement of [125I]A-312110 binding with the same K+ channel openers to the SUR2B exon 17- cells showed excellent correlation to those measured in guinea pig bladder. This study supports the notion that KATP channels containing SUR2B exon 17- represent a major splice variant expressed in urinary bladder smooth muscle.


http://www.sciencedirect.com/science/article/B6T1J-3RM6MFJ-N/2/5f50a5615a0bdd5231f6a6c281a7b764

We previously reported that tumor necrosis factor-[alpha]/cachectin suppresses lipoprotein lipase activity and its gene expression in brown adipocytes differentiated in culture. Recent evidence suggests that the effect of TNF-[alpha] over various cells is related to the enhanced production of nitric oxide (NO). The present study examined whether the suppressive effect of TNF-[alpha] on lipoprotein lipase activity is mediated by production of NO in the brown adipocytes. A reverse transcription-polymerase chain reaction (RT-PCR) assay revealed that TNF-[alpha] caused a concentration- and time-dependent expression of inducible NO synthase in brown adipocytes. Increasing concentrations of TNF-[alpha] (0.5-50 ng/ml) for 24 h resulted in a concentration-dependent decrease in lipoprotein lipase activity with reciprocal increase in nitrite production in the medium. The suppressive effect of TNF-[alpha] on lipoprotein lipase activity was significantly prevented by NO synthase inhibitors, NG-nitro-arginine methyl ester (-NAME) and aminoguanidine, but not by -NAME, an inactive isomer. Furthermore, 8-bromoguanosine 3’,5’-cyclic monophosphate, cell permeant cGMP, suppressed lipoprotein lipase activity and 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one, a selective inhibitor for soluble guanylate cyclase, restored the TNF-[alpha]-suppressed lipoprotein lipase activity. These results suggest that TNF-[alpha] stimulates brown adipocytes to express inducible NO synthase, followed by production of NO, which in turn mediates the suppressive effect of TNF-[alpha] on lipoprotein lipase activity. The effect of NO is mediated, at least partly, through production of cGMP.


http://www.sciencedirect.com/science/article/B6T1J-3SVS0NN-24/2/ad8e47a36097c9191a604cba723e5128

Activation of cardiac [alpha]1-adrenoreceptors has a number of physiological effects. Ascribing these effects to a specific [alpha]1-adrenoreceptor subtype first requires the elucidation of the subtypes that are present in the tissue of interest. In the present study, mRNA transcripts for the [alpha]1A, [alpha]1B and [alpha]1D-adrenoreceptor subtypes were detected in cultured neonatal rat cardiac myocytes, using reverse transcriptase-polymerase chain reaction analysis. However,
binding sites for only the [alpha]1A and [alpha]1B-adrenoreceptor subtypes were detected in cultured neonatal rat cardiac myocytes, using competition binding analysis with a variety of [alpha]1 selective receptor antagonists. Phenylephrine-stimulated phosphatidylinositol hydrolysis was inhibited by [alpha]1 selective receptor antagonists with affinities consistent with the [alpha]1A-adrenoreceptor subtype, whereas phenylephrine-induced activation of the mitogen activated protein kinase cascade was inhibited by these same antagonists with affinities more closely resembling the [alpha]1B-adrenoreceptor subtype. In the case of both signaling pathways, the [alpha]1D selective receptor antagonist, BMY 7378, exhibited affinities suggestive of the relative absence of a [alpha]1D-adrenoreceptor subtype. Thus, despite the presence of mRNA transcripts for all three [alpha]1-adrenoreceptor subtypes, only the [alpha]1A and [alpha]1B-adrenoreceptor subtypes were expressed and functionally coupled at detectable levels in neonatal rat cardiac myocytes. Of particular interest, phenylephrine-induced activation of the mitogen activated protein kinase cascade appears to be mediated by a subtype resembling most closely the pharmacological profile of the [alpha]1B-adrenoreceptor subtype.

European Journal of Pharmacology: Environmental Toxicology and Pharmacology (1)


http://www.sciencedirect.com/science/article/B73F5-47S65Y3-1R/2/dbc01adb13578c67cefacce68cd4a130

Expression of human cytochrome P450 (CYP) in heterologous cells is a means of specifically studying the role of these enzymes in drug metabolism. The complete cDNA encoding CYP3A4 (PCN1) was inserted into an expression vector containing the strong myeloproliferative sarcoma virus promoter in combination with the enhancer of the cytomegalovirus and stably expressed in V79 Chinese hamster cells. The presence of genomically integrated CYP3A4 cDNA cell clones was confirmed by polymerase chain reaction analysis. Transcription was detected by reverse transcribed polymerase chain reaction analysis. Functional expression could be demonstrated by conversion of testosterone to the specific 6[beta]-hydroxylated product. In recombinant V79 cells expressing CYP3A4 about 6% of the substrate was converted to 6[beta]-hydroxytestosterone. The metabolism of two dopaminergic ergot derivatives was investigated in live recombinant V79 cells. Both lisuride and terguride were monodeethylated.

European Journal of Pharmacology: Molecular Pharmacology (5)


http://www.sciencedirect.com/science/article/B73F6-478BPR3-
The expression of the [alpha]1C-adrenoceptor subtype in human and rabbit blood vessels has been analyzed using the reverse transcriptase/polymerase chain reaction technique (RT/PCR). The 20 bp primers employed were designed from the bovine [alpha]1C-adrenoceptor and flank a least conserved region -- the putative third cytoplasmic loop. RT/PCR products generated from rabbit and human brain mRNA both had 93% homology to the bovine [alpha]1C-adrenoceptor and were used as species and subtype specific probes in Southern blot analysis of vascular RT/PCR products. Poly A+ RNA was purified from the human saphenous vein and rabbit aorta, renal, pulmonary and central ear arteries and amplified by RT/PCR. Size analysis by agarose gel electrophoresis, together with Southern hybridization of the resulting cDNA products confirm the expression of the [alpha]1C-adrenoceptor in these vessels.


http://www.sciencedirect.com/science/article/B73F6-47STG39-1P/2/a7ff7de9f8056974e59d0ec508b27f03b

Rat glutamate receptors have been shown to be expressed as two developmentally regulated, alternatively spliced isoforms. We have investigated the expression of these isoforms of GluRA and GluRB in the human hippocampus. The expression pattern of the mRNAs coding for these subunits does not correspond to that in the rat hippocampus, both isoforms being preferentially expressed in the dentate gyrus and CA1 regions, with lower expression in CA3, with the...
exception of GluRB flop, where hybridization in CA3 is only lower than in dentate gyrus. Cloning of cDNA from human frontal cortex has also revealed that the two isoforms of human GluRB have virtual nucleotide sequence identity with the alternative exons in the rat, confirming the usefulness of oligonucleotides complementary to the rat cDNAs as probes for these receptor subunits in human neuropsychiatric disorders.


http://www.sciencedirect.com/science/article/B73F6-47STG5J-2T2/2/6c8e0609e74e5a639b2ef87e9cc04c0

Chronic administration (twice a day for three days and on the morning of the fourth day) of SR 46349B (trans-4-[(3Z)-3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl]phenol hemifumarate) (10mg/kg, orally), a selective 5-HT2 receptor antagonist, caused 24 h later a marked increase (+ 42%) of the maximum binding capacity of [3H]ketanserin in rat brain cortical membranes without change in its affinity constant. Further, administration of the 5-HT2 receptor agonist, (+/-)-DOI((+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) (1 mg/kg, i.p.), produced in chronic SR 46349B treated rats a significant increase in the amount of [3H]-inositol phosphate compared to corresponding controls. In addition, subacute administration of SR 46349B caused a 2-fold increase in the head-twitch response to (+/-)-DOI (0.5 mg/kg, i.p.). This enhanced response was blocked by an acute administration of ritanserin (6-{2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl}-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one) (10 mg/kg). Finally, a significant enhancement (+ 29%) of 5-HT2 receptor mRNA levels was observed in the cortex. Taken together, these data showed that an up-regulation of 5-HT2 receptors occurred in rats following repeated treatment with a selective 5-HT2 receptor antagonist. The effects of SR 46349B on 5-HT2 receptors might implicate pre-translational regulation.


http://www.sciencedirect.com/science/article/B73F6-47S63NN-1W/2/77800af4a4249ba508413efa378cb4cf

Benzodiazepines modulate [gamma]-aminobutyric acid (GABA)-evoked chloride currents through a specific binding site at the GABAA receptor-chloride channel complex. The heterogeneity of diazepam-sensitive benzodiazepine binding sites (type I and type II) has been identified by pharmacological approaches both with native receptors and recombinant receptors coexpressing [alpha], [beta] and [gamma] subunits. In addition, two distinguishable diazepam-insensitive benzodiazepine sites are found, spatially distributed between cerebral cortical and cerebellar regions. Coexpression of [alpha]6 with [beta]2 and [gamma]2L subunits creates a pharmacologically similar benzodiazepine receptor to the diazepam-insensitive site observed in cerebellum, however, there is no evidence regarding the possible subunit combination forming the DI site in cerebral tissues. Here we report the cloning of the human [alpha]4 cDNA and its pharmacology by coexpression of this [alpha]4 subunit with [beta]2 and [gamma]2L subunits. This recombinant receptor complex showed a high affinity for the previously described benzodiazepine partial agonist bretazenil, the pyrazoloquinoxine compounds CGS-9895 and CGS-9896, as well as the inverse agonists DMCN (methyl 6,7-dimethoxy 4-ethyl-[beta]-carboline-3-carboxylate) and Ro15-4513 as determined by [3H]Ro15-4513 binding. However, it is insensitive to the benzodiazepine type I selective compounds CL218,872 (3-methyl-6-[3-(trifluoromethyl)phenyl]-
1,2,4-triazolo[4,3-b]pyridazine) and zolpidem as well as the benzodiazepine full agonists diazepam, halazolam and midazolam. In addition, the benzodiazepine receptor ligands DMCM, [beta]-CCE ([beta]-carboline-3-carboxylate ethyl ester), [beta]-CCM ([beta]-carboline-3-carboxylate methyl ester), FG-7142, CGS-9895 and CGS-9896 showed 7 to 10 times higher affinity for [alpha][4][beta][2][gamma][2]L than for [alpha][6][beta][2][gamma][2]L. The pharmacology of the [alpha][4][beta][2][gamma][2]L receptor complex appears to resemble those of the diazepam-insensitive site found in the cerebral cortex. Our study thus suggests that this subpopulation of diazepam-insensitive GABAA receptors may be composed of [alpha][4][beta][2][gamma][2]L subunits.

European Journal of Soil Biology (1)


http://www.sciencedirect.com/science/article/B6VR7-4593YCJ-F/2/df594d8506e8806abdf591459d00b104

Forty selected bacterial isolates of the faecal microbial community in an African millipede belonging to the family Spirostreptidae were characterized by amplified ribosomal DNA-restriction analysis (ARDRA) and by sequencing either the first 500 bp or almost the entire length of the 16S rRNA gene. Sequence data show that the faecal microbial community investigated is dominated by members of the class Actinobacteria with most of the strains belonging to the genera Rhodococcus, Cellulomonas and Leifsonia. In certain cases, low similarity values to the previously described 16S rDNA sequences suggest that these strains presumably constitute new species within the Actinobacteria.

European Journal of Vascular and Endovascular Surgery (2)


http://www.sciencedirect.com/science/article/B6WF5-4F02KXS-1/2/3ad9306be3f51787308c883e1fbb3906

ObjectiveSerological studies have suggested that one of the risk factors for aneurysm development is C. pneumoniae infection. The purpose of this study was to evaluate whether there is an association between the presence of C. pneumoniae DNA in aneurysms and ruptured Abdominal Aortic Aneurysms. MethodsAortic walls were collected consecutively from 30 patients with intact AAA, 16 patients with ruptured AAA and 19 healthy organ donors (control). Purified DNAs from all aortas were analyzed for the presence of C. pneumoniae DNA in parallel by
polymerase chain reaction-enzyme immunoassay (PCR-EIA) and agarose gel electrophoresis. PCR-EIA has a high sensitivity in detecting low DNA copy number in clinical atherosclerotic samples.

Results C. pneumoniae DNA was detected more frequently in patients with aneurysms, particular with ruptured aneurysms. The incidence of positive C. pneumoniae DNA was 73.3% in intact AAA and 10.5% in control aortas, with the highest frequency in ruptured AAA (100%) (pConclusion)

Giving the high specificity and sensitivity of PCR-EIA, these findings support the association of C. pneumoniae in the pathogenesis of aneurysm development, growth and rupture.


http://www.sciencedirect.com/science/article/B6WF5-4DFT2FH-3/2/1e3dd3849d009115f10bd4bb32d5936

Objectives Photodynamic therapy (PDT) is a promising strategy to limit restenosis. PDT depletes the resident cells from the vessel wall without adventitial cell ingrowth. This study was undertaken to further explore the mechanisms by which PDT of matrix acts on key mechanisms in the development of restenosis.

Materials and Methods Control and PDT-treated collagen type-I matrix gels were prepared. Thereafter, untreated human fibroblasts were seeded on matrix gels (n=12). Fibroblast proliferation and invasive migration were quantified by calibrated phase contrast microscopy. Fibroblast bFGF and TGF-[beta]1 mRNA expression were analyzed using a quantitative real-time reverse transcription polymerase chain reaction.

Results Fibroblast proliferation on PDT-treated matrix gels was reduced by 30 and 76% after 3 and 7 days, respectively (3 days: P[less-than or equal to]0.01, 7 days: P[less-than or equal to]0.001). PDT of matrix gels led to a 47% reduction of migration after 3 days and 51% after 7 days (P[less-than or equal to]0.001). PDT led to a 77% reduction of fibroblast TGF-[beta]1 mRNA (P[less-than or equal to]0.02) and to a 79% reduction of bFGF mRNA (P[less-than or equal to]0.03). Conclusions PDT of matrix-induced reduction of bFGF and TGF-[beta]1 mRNA levels may be important mechanisms of reducing fibroblast proliferation and invasive migration and thus the development of restenosis. These newly identified mechanisms highlight PDT’s pleiotropic effects on the vessel wall and its potential clinical value.


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European Neuropsychopharmacology (1)


http://www.sciencedirect.com/science/article/B6T26-4D34NXS-1/2/58c88bc0b9c9c3f3cc2128fb55560b09

The symptoms of attention deficit hyperactivity disorder (ADHD) can be treated with methylphenidate (MPH), a potent blocker of dopamine transporter (DAT). The homozygosity of the 10-repeat allele at the DAT gene (DAT1) seems to be associated with a poor response to MPH in children with ADHD. In the present study, we investigated the association between DAT
density using I-123-N-(3-iodopropen-2-yl)-2[beta]-carbomethoxy-3beta-(4-chlorophenyl)tropane [123I]IPT single photon emission computed tomography (SPECT) and the homozygosity for 10-repeat allele at DAT1 and response to MPH in Korean children with ADHD. Eleven drug-naive children with ADHD were included in the study and treated with MPH for about 8 weeks. After the genotyping and SPECT were performed, we compared DAT density between ADHD children with and without the homozygosity for 10-repeat allele at DAT1 and investigated the correlation between the homozygosity for 10-repeat allele and response to MPH. ADHD children with 10/10 genotype (n=7) had a significantly greater increase of the DAT density in basal ganglia than the children without 10/10 genotype (n=4). We found that while only 28.6% (2/7) of the subject with 10/10 genotype showed good response to MPH treatment, 100% (4/4) of the subjects without 10/10 genotype showed good response to MPH treatment. Our findings support an association between homozygosity for 10-repeat allele at DAT1 and the DAT density assessed in vivo and correlation between the homozygosity for 10-repeat allele and poor response to MPH.

European Urology (2)


http://www.sciencedirect.com/science/article/B6X10-4CJV6F1-1/2/55505bed692ad3cb728ae5f0eeec02e39

Objectives: Alpha-1-antitrypsin ([alpha]1-antitrypsin) is a major protease inhibitor controlling tissue degradation. Reduced [alpha]1-antitrypsin levels could result in a change of collagen metabolism. Previous studies have described decreased [alpha]1-antitrypsin levels in patients with Peyronie's disease. However, only a small number of patients were analyzed, and the reason for the decreased [alpha]1-antitrypsin levels remained unclear. This study investigated prospectively the levels of [alpha]1-antitrypsin in patients with Peyronie's disease, as well as genetic variation in the coding region of the [alpha]1-antitrypsin gene. Methods: [alpha]1-antitrypsin levels were determined prospectively in 94 patients with Peyronie's disease and compared to healthy controls. Analysis of the [alpha]1-antitrypsin gene (S, Z variants; single nucleotid polymorphisms [SNPs]: T-395A, M2, M3, G6118A) was done in 141 Peyronie's patients including 43 patients with investigated [alpha]1-antitrypsin serum levels and compared to healthy controls. Results: In patients with Peyronie's disease, the [alpha]1-antitrypsin levels seemed to be decreased significantly compared to healthy controls. However, in the age matched approach no significant differences occurred. Moreover, a significant (p<0.05) association between Peyronie's disease and decreased [alpha]1-antitrypsin levels. Low [alpha]1-antitrypsin levels in Peyronie's patients are, rather, an age-related phenomenon, as revealed by the comparison with aged matched healthy controls. The decrease of the [alpha]1-antitrypsin serum level with increasing age has not been described before.


http://www.sciencedirect.com/science/article/B6X10-479M4FH-2/2/a8c457119184bf55a9f5f6441a172325
Objectives: Osteocalcin is a vitamin-K dependent protein which is related to the metabolism of bone and calcium. The formation or progression of prostate cancer is presumed to be associated with the osteocalcin gene. The most frequently seen polymorphism is HindIII which is located at the promoter region. HindIII is therefore a possible genetic marker in the search for the association between prostate cancer and normal control subjects.

Methods: In our study, a normal control group of 132 healthy people and 96 patients with prostate cancer were examined. The polymorphism was seen following polymerase chain reaction (PCR) based restriction analysis.

Results: The result revealed significant differences between normal individuals and cancer patients (p=0.034) and the distribution of the "CC" homozygote in the control group was higher than that in the patient group. No statistical differences were found in clinical staging and grading. The 54 patients who received hormone therapy were further categorized into response and non-response groups, statistical differences between these two groups were revealed (p=0.007, Fisher's exact test).

Conclusions: Based on our results, we conclude that the HindIII polymorphism of the osteocalcin gene is a suitable genetic marker of prostate cancer which can be used in the prediction of the outcome of patients who receive hormone therapy.

Evid. Based Complement. Altern. Med.  (1)


http://ecam.oupjournals.org/cgi/content/abstract/neh077v1

We have established an allergic dermatitis model in NC/Nga mice by repeated local exposure of mite antigen for analyzing atopic dermatitis. We examined how four Kampo medicines, Juzen-taiho-to, Hochu-ekki-to, Shofu-san and Oren-gedoku-to, on the dermatitis model to obtain basic information on their usefulness for treating atopic dermatitis. Mite antigen (Dermatophagoides farinae crude extract) solution at a concentration of 10 mg/ml was painted on the ear of NC/Nga mice after tape stripping. The procedure was repeated five times, at 7 day intervals. An apparent biphasic ear swelling was caused after the fourth and fifth antigen exposures with elevated serum IgE levels and accumulation of inflammatory cells. In the cervical lymph nodes and ear lobes, the five procedures of antigen exposure induced interleukin-4 mRNA expression but reduced interferon-(gamma) mRNA expression. Oral administration of all four Kampo medicines inhibited the formation of ear swelling and inflammatory cell accumulation. Juzen-taiho-to and Hochu-ekki-to apparently prevented the elevation of serum IgE level. Furthermore, the four Kampo medicines showed a tendency to prevent not only the increase in interleukin-4 mRNA expression but also the decrease in interferon-(gamma) mRNA expression. The present results indicate that Juzen-taiho-to, Hochu-ekki-to, Shofu-san and Oren-gedoku-to may correct the Th1/Th2 balance skewed to Th2, and this activity helps inhibit dermatitis in NC/Nga mice. The ability of the Kampo medicines to correct the Th1/Th2 balance seems to underlie their effectiveness in treating of atopic dermatitis.

Experimental and Molecular Pathology  (5)

http://www.sciencedirect.com/science/article/B6WFB-4CMHSX3-1/2/8a93df03fa40c34bb2a5a0d958c87da

We have investigated the incidence of the C677T and A1298C methylene tetrahydrofolate reductase (MTHFR) gene single nucleotide polymorphisms (SNPs) in the South Indian Tamil Nadu population with a total number of 72 individuals. The MTHFR genotyping was performed using the polymerase chain reaction followed by restriction enzyme analysis. Homozygosity for the MTHFR A1298C SNP was detected in 15.3% (11/72) of the individuals tested, and 47.2% (34/72) were heterozygous for this SNP. Homozygosity for the C677T MTHFR SNP was detected in 1.38% (1/72), and the frequency of the C677T heterozygotes was 18.1% (13/72). When we analyzed the combined frequency of the two SNPs, the frequency of double heterozygosity was 19.6%, and the frequency of double homozygosity was completely absent among the study group. The 'C' allele frequency for MTHFR A1298C was 0.389, and the 'T' allele frequency for C677T mutation was 0.104. Out of the 72 individuals included in the study, 52 were acute myocardial infarction (AMI) patients and 20 were healthy individuals with no documented history of heart disease. The results of this study indicate that the MTHFR A1298C SNP is more prevalent among the Tamilians when compared to the MTHFR C677T SNP, suggesting a possible role of MTHFR A1298C in the pathogenesis of heart diseases.


http://www.sciencedirect.com/science/article/B6WFB-48M7RHB-1/2/4d34d2a03ef15be2d5fa4eaddbd19b483

Dendritic cells (DCs) consist of a heterogeneous population of hematopoietic cells characterized by their unique dendritic morphology, their efficient antigen-presenting capability to activate naive CD4+ and CD8+ T cells, and their lack of lineage specific markers. Functional properties comparing umbilical cord blood monocyte-derived and umbilical cord blood stem cell-derived DCs have not yet been investigated. CD14+ monocytes and CD34+ stem cells were isolated from human umbilical cord blood and were induced to differentiate into dendritic cells using 100 ng/mL granulocyte-macrophage colony stimulating factor (GM-CSF), 25 ng/mL IL-4, 2.5 ng/mL tumor necrosis factor-[-alpha] (TNF-[-alpha]), 100 ng/mL GM-CSF, 25 ng/mL stem cell factor, and 2.5 ng/mL TNF-[-alpha], respectively. Flow cytometric analysis revealed that the 14-day-old dendritic cells were CD80+, CD86+, CD83+, CD54+, CD1a+, CD11b+, CD11c+, HLA-DR+, CD34+, CD3-, CD19-, CD14-, and CD16-. Reverse transcription polymerase chain reaction was employed to detect expression of mRNA for CD80 and CD86. Differentiating monocytes initially expressed CD86 while CD80 appeared on day 2. Differentiating stem cells expressed CD80 and CD86 on day 2 of culture. The surface expression of CD80 and CD86 was studied over the course of differentiation. Mixed lymphocyte reaction was employed to evaluate the two types of lineage-derived DCs. Prior to the functional assay, CD14+ and CD34+ derived DCs were stimulated for 18 h with 0.1 mg/mL and 1.0 mg/mL E. coli lipopolysaccharide, respectively. Monoclonal antibodies (mabs) to CD80 and CD86 were used to assess their costimulatory roles. A decrease of stimulation as depicted by decreased T cell activation was significant with mabs to both CD80 and CD86 on monocyte-derived DCs while only mabs to CD86 induced decreased T cell activation by stem cell-derived DCs. The varied functional role of CD80 and CD86 costimulatory molecules is associated with DC differentiation from distinct cord blood isolated hematopoietic lineages. These studies demonstrate that DC association with distinct hematopoietic lineages is of relevance in transplantation and vaccine therapies.

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Whole blood samples of known methylene tetrahydrofolate reductase (MTHFR) genotypes from 24 individuals were examined at site C677T. Their amplified DNA products were assessed by two-color fluorescence cross-correlation measurements and agarose gel electrophoresis/capillary gel electrophoresis. DNA subpopulations were identified which were not associated with the proper genotype by primer combinations and cycling conditions called multiplexes. We confirmed that DNA analysis by two-color fluorescence cross-correlation measurements allowed the detection of fluorescence signals specifically associated with the proper genotypes in a mixture of amplified nontarget DNA molecules without DNA sizing. The measurement approach does not require complex, follow-up mathematical analysis and is applicable to any single nucleotide polymorphisms. The simple immunogenetic model showed how the approach works to reveal specific DNA target by preventing detection of nontarget DNA. Under those experimental conditions, a new ultrasensitive, and specific method for clinical immunologists is born.

Members of the inhibitor of apoptosis protein (IAP) family, including survivin, have been reported to be expressed in many tumors. However, their expression in esophageal cancer has not been clarified completely. We investigated the expression of mRNA for IAP family proteins in samples from esophageal cancers and their adjacent normal mucosa tissues by real-time quantitative RT-PCR. The survivin expression in esophageal cancer was significantly higher than that in normal mucosa (P < 0.05). Immunohistochemical staining demonstrated cytoplasmic as well as nuclear expression of survivin in esophageal cancer, and further, in situ hybridization analysis demonstrated cytoplasmic expression of mRNA for survivin. The results suggest that the expression of IAP family proteins, especially survivin, may be associated with the biological character of esophageal cancer, such as apoptosis.

Experimental and Toxicologic Pathology


Brown Norway (BN) and Fischer 344 (F344) rats were exposed to aerosol of 1% ovalbumin (OVA) solution for 30 min at 1 week after the second sensitization with 1 mg of OVA at 2-week intervals. Changes in the histology and expression of cytokines and chemokines in the lung were examined for up to 96 h after the exposure. The lung weight significantly increased in BN rats but not in F344 rats. Histologically, in the lung of BN rats, multiple foci of hemorrhage in the alveolar space with infiltration of eosinophils and macrophages in the surrounding alveolar septa were first observed. Thereafter, granulomatous lesions developed in the preexisting hemorrhagic foci, finally resulting in formation of multiple eosinophilic granulomas. On the other hand, in F344 rats, infiltration of eosinophils and macrophages was observed around the vessels and bronchi. Thereafter it progressed gradually, resulting in mild thickening of alveolar septa. The levels of Th1- (interferon-[gamma] and interleukin 2 (IL-2)) and Th2-related cytokines (IL-4 and IL-5) and chemokines (eotaxin and monocyte chemotactrant protein-1) mRNAs measured by reverse transcription-polymerase chain reaction method were elevated in the lung of both strains, and the levels were higher in BN rats than in F344 rats. These results suggest that BN rats are more sensitive to OVA-sensitization/inhalation than F344 rats and that the difference in the severity of lung lesions between BN and F344 rats may reflect the difference in the expression levels of cytokines and chemokines between these two strains.

Experimental Biology and Medicine

Reproduction and development are highly dependent on apoptosis to balance the proliferation that necessarily occurs during these processes. How the absence of two apoptotic factors in mice would affect reproduction and development was examined. Given previous reports of increased neural tube defects in p53-/- female fetuses, decreased fertility in gld female mice, and altered spermatogenesis in both p53 and gld male mice, the possibility that these phenotypes might be enhanced by the elimination of a second apoptotic factor was investigated. The reproductive vigor and the health of offspring were monitored during the production of the new double-deficient strain (FasL-/-p53-/-) for any changes from the reported phenotypes. Thus, any unusual phenotypes that could lead to new models for studying mechanisms of health and disease would be identified. Double-deficient male offspring appeared healthy and occurred at expected frequencies. Additionally, spermatogenesis and male fertility were unaffected by the gene deficiencies. On the other hand, FasL+/+p53-/- and FasL-/-p53-/- female mice were susceptible to increased malformations and post-natal death. These abnormalities were consistent with previous reports of neural tube defects in p53-/- female mice. Fertility rates were also significantly decreased in p53-/- female mice that lived to be adults, an observation not previously reported. Finally, the absence of both FasL and p53 led to dystocia in pregnant female mice, suggesting that the two genes play complementary roles in parturition. Therefore, although male mouse development and reproduction remained unaffected by p53 and FasL deficiencies, female mouse development was adversely affected by the absence of p53, and no live litters were born to female mice with the combined absence of both FasL and p53. In this report, we suggest a potential mechanism involving corpora luteal regression to explain this defect in parturition in FasL-/-p53-/- female mice.


Flavonoids isolated from cocoa have biological activities relevant to oxidant defenses, vascular health, tumor suppression, and immune function. The intake of certain dietary flavonoids, along with other dietary substances such as tocopherols, ascorbate, and carotenoids, is epidemiologically associated with a reduced risk of cardiovascular disease. Flavonoids have also been shown to modulate tumor pathology in vitro and in animal models. We took advantage of the conserved sequences found in tyrosine kinases to study the influence of cocoa fractions and controls on gene expression. We report that the pentameric procyanidin (molecular weight of 1442 daltons) fraction isolated from cocoa was a potent inhibitor of tyrosine kinase ErbB2 expression, a receptor important in angiogenesis regulation. Consistent with this primary observation, the cocoa flavonoid fraction also suppressed human aortic endothelial cell (HAEC) growth and decreased expression of two tyrosine kinases responsive to ErbB2 modulation, namely VEGFR-2/KDR and MapK 11/p38(β)2. These inhibitory effects were observed when HAECs were treated with the flavonol fraction (molecular weight 280 daltons) isolated from cocoa, which comprise the structural subunits from which the procyanidin flavonoid subclass is biosynthetically constructed. Down-regulation of ErbB2 and inhibition of HAEC growth by cocoa procyanidins may have several downstream implications, including reduced vascular endothelial growth factor (VEGF) activity and angiogenic activity associated with tumor pathology. These results suggest specific dietary flavonoids are capable of selectively inhibiting ErbB2 and therefore may offer important insight into the design of therapeutic agents that target tumors overexpressing ErbB2.

http://www.ebmonline.org/cgi/content/abstract/228/3/261

Traumatic brain injury (TBI) causes excess release of neurotransmitters, such as glutamate, and increases intracellular calcium levels. Elevated levels of calcium, and perhaps other intracellular second messengers, as a result of TBI can alter the expression of many genes. The protein products of some of these genes may be signals for TBI-associated memory dysfunction. Therefore, identification of genes whose expression is altered after TBI in the hippocampus, a structure in the medial temporal lobe that plays a critical role in memory formation and storage, and elucidation of the role(s) of their protein products may shed light on the molecular mechanisms underlying TBI-elicited memory dysfunction. The prodynorphin gene is expressed in hippocampal granule cells, and its expression has been reported to be enhanced as a result of elevated intracellular calcium. The prodynorphin protein is proteolytically cleaved to generate multiple dynorphin peptides, which can modulate neurotransmitter release through the activation of presynaptic (kappa) opioid receptors. In this study, we report that 1) TBI transiently increases prodynorphin mRNA in the hippocampus, 2) dynorphin peptide immunoreactivity is enhanced for up to 24 hr after TBI and 3) intracerebroventricular infusion of the (kappa) receptor antagonist nor-binaltorphimine (nor-BNI) impairs subsequent performance in a spatial memory task. These results suggest that dynorphin action may serve a beneficial role after TBI.


http://www.ebmonline.org/cgi/content/abstract/227/1/26

Metallothioneins (MT) are low-molecular-weight, heat-stable, cysteine-rich proteins with four isoforms. MT-I and MT-II are ubiquitous and are induced by oxidative, physical, and chemical stress. MT-I is an efficient scavenger of superoxide (*O2) and hydroxyl ion (OH-). We have demonstrated that *O2 and hypohalous acid can cause an increase in glomerular albumin permeability (Palb) in vitro. The purpose of this study was to document the protective effect of MT gene product on the *O2-mediated increase in Palb. Glomeruli from Sprague-Dawley rats in 4% BSA medium were incubated for 4 hr at 37{degrees}C in duplicate tubes. Each set contained glomeruli alone or with 5 {micro}M Cd++, 0.3 mM Spermine-NONOate (NO donor), 0.3 mM Sulfo-NONOate (nitrous oxide donor), 0.6 mM SNP (nonspecific NO donor) and SNP + carboxy-PTIO (10 mg/ml). After incubation, one set of tubes was used to isolate total RNA for the measurement of the mRNA levels of MT-I by reverse transcriptase polymerase chain reaction (RT-PCR). Duplicate tubes were incubated for an additional 10 min with 10 nM of *O2, and Palb was measured using video microscopy. RT-PCR of total RNA from Cd++ and Spermine-NONOate treated glomeruli revealed a 2-fold induction of MT-I expression at the mRNA level,*O2 caused a significant increase in Palb (0.8 {+/-} 0.06 vs. control 0.0 {+/-} 0.12, P < 0.05) and induction of MT-I in glomeruli by Cd++ or by Spermine-NONOate blocked this effect (0.21 {+/-} 0.12 and 0.24 {+/-} 0.19, respectively, P < 0.05 vs. *O2). In contrast, Sulfo-NONOate and SNP did not induce mRNA for MT-I in glomeruli and did not provide protection against *O2-mediated increase in Palb. We conclude that MT-I gene products may play an important role in protecting the glomerular filtration barrier from the injury induced by reactive oxygen species in immune and/or nonimmune renal diseases.

Monocytes play key roles both in innate and adaptive antigen-specific immunity and they constitute critical components of the immune responses. Although most of the monocyte-derived cytokines exhibit proinflammatory functions in vivo, heme oxygenase-1 (HO-1), an inducible heme-degrading enzyme, exerts potent anti-inflammatory effect through production of carbon monoxide and bilirubin. We compared HO-1 production by monocytes in vivo in various acute inflammatory illnesses and in normal controls. Freshly isolated monocytes produced little HO-1 as detected by immunohistochemistry, but it was rapidly induced in vitro upon stimulation. HO-1 production by monocytes was selective because it was not induced in other leukocyte populations, including granulocytes and lymphocytes. Monocytes from acute inflammatory illnesses, such as Kawasaki disease and acute infectious diseases, viral or bacterial, produced significant levels of HO-1, as detected by flow cytometry, immunohistochemistry, and reverse transcription polymerase chain reaction. Quantitative analysis of HO-1 mRNA expression by real-time polymerase chain reaction revealed that monocytes from controls exhibited low, but significant levels of HO-1 mRNA, indicating that circulating monocytes produce HO-1 constantly, in response to basal level of oxidative stress encountered daily. Significantly elevated HO-1 mRNA levels seen in acute inflammatory illnesses suggest that monocyte HO-1 production serve as potent anti-inflammatory agent to control excessive cell or tissue injury in the presence of oxidative stress and cytokinemia.

Experimental Cell Research (9)


The protein dlk, encoded by the Dlk1 gene, belongs to the Notch epidermal growth factor (EGF)-like family of receptors and ligands, which participate in cell fate decisions during development. The molecular mechanisms by which dlk regulates cell differentiation remain unknown. By using the yeast two-hybrid system, we found that dlk interacts with Notch1 in a specific manner. Moreover, by using luciferase as a reporter gene under the control of a CSL/RBP-Jk/CFB-1-dependent promoter in the dlk-negative, Notch1-positive Balb/c 14 cell line, we found that addition of synthetic dlk EGF-like peptides to the culture medium or forced expression of dlk decreases endogenous Notch activity. Furthermore, the expression of the gene Hes-1, a target for Notch1 activation, diminishes in confluent Balb/c14 cells transfected with an expression construct encoding for the extracellular EGF-like region of dlk. The expression of Dlk1 and Notch1 increases in 3T3-L1 cells maintained in a confluent state for several days, which is associated with a concomitant decrease in Hes-1 expression. On the other hand, the decrease of Dlk1 expression in 3T3-L1 cells by antisense cDNA transfection is associated with an increase in Hes-1 expression. These results suggest that dlk functionally interacts in vivo with Notch1, which may lead to the regulation of differentiation processes modulated by Notch1 activation and signaling, including adipogenesis.
Activity of the independently regulated human c-myc P0 promoter has been associated with the undifferentiated status of leukemia cells as well as the hormone-independent proliferation of breast cancer cells. The P0 transcript is distinguished from the predominant P1 and P2 c-myc mRNAs by an ~639-nucleotide extension of the 5'-untranslated region. We hypothesized that this complex 5'-untranslated RNA sequence unique to the P0 transcript may contribute significantly to the composite regulation of the c-myc locus and that enforced intracellular synthesis of the isolated P0 5'-UTR, out of its native sequence context, might amplify or dominantly interfere with its normal regulatory function. Human tumor (HeLa) cells in which the isolated P0 5'-UTR was ectopically expressed displayed a dramatic decrease in anchorage-independent proliferation. Furthermore, P0 5'-UTR-expressing HeLa cells failed to form tumors when inoculated into SCID mice. This loss of tumorigenicity was associated with increases in levels of the c-Myc1 (p67) and c-Myc2 (p64) proteins and a 3- to 5-fold elevation of spontaneous apoptotic index. These results demonstrate that an isolated 5'-untranslated RNA sequence can be attributed potent in trans gene-regulatory and phenotype-altering capabilities and that extrinsic alterations in c-myc regulation can be utilized to reestablish the natural proapoptotic (tumor suppressor) activities associated with this protooncogene.

Among the more than 30 different human proteins of the cytokeratin (CK) group of intermediate filament (IF) proteins, the significance of the epidermal polypeptide CK 2 (Moll et al., 1982, Cell 31, 11-24) has been repeatedly questioned in the literature. Here, we show, by in vitro translation and protein gel electrophoresis, that human epidermis from various body sites does indeed contain relatively large amounts of mRNA encoding a distinct polypeptide comigrating with native epidermal CK 2. We also report the isolation of a cDNA clone encoding the complete sequence of CK 2, which is a type II CK different from--but related to--epidermal CKs 1 and 5 on the one hand and corneal CK 3 on the other. The mRNA of ~2.6 kb encodes a polypeptide of 645 amino acids and Mr 65,852, in good agreement with the value of 65.5 kDa previously estimated from gel electrophoresis. This human CK, the largest so far known, displays several features typical of CKs of stratified epithelia, including numerous repeats of glycinerich tetrapeptides in the head and tail domains. Northern blot and in situ hybridizations have shown that CK 2 is expressed strictly suprabasally, usually starting in the third or fourth cell layer of epidermis, and this was confirmed at the protein level by immunohistochemistry using CK 2-specific antibodies. The protein has been detected as a regular epidermal component in skin samples from different body sites, albeit as a minor CK in "soft skin" (e.g., breast nipple, penile shaft, axilla), but not in foreskin epithelium and in other epithelia, in squamous metaplasias and carcinomas, or in cultured cell lines derived therefrom. We propose that CK 2 is a late cytoskeletal IF addition synthesized during maturation of epidermal keratinocytes which probably contributes to terminal cornification.
HIPK2 is a member of a novel family of nuclear serine-threonine kinases identified through their ability to interact with the Nkx-1.2 homeoprotein. The physiological role of these kinases is largely unknown, but we have recently reported on the involvement of HIPK2 in the induction of apoptosis of tumor cells after UV stress through p53 phosphorylation and transcriptional activation. Here, we demonstrate that the chemotherapeutic drug cisplatin increases HIPK2 protein expression and its kinase activity, and that HIPK2 is involved in cisplatin-dependent apoptosis. Indeed, induction of HIPK2 and of cell death by cisplatin are efficiently inhibited by the serine-threonine kinase inhibitor SB203580 or the transduction of HIPK2-specific RNA-interfering molecules. HIPK2 gene silencing efficiently reduces the p53-mediated transcriptional activation of apoptotic gene promoters as well as apoptotic cell death after treatment with cisplatin. These findings, along with the involvement of p53 phosphorylation at serine 46 (Ser46) in the transcriptional activation of apoptotic gene promoters, suggest a critical role for HIPK2 in triggering p53-dependent apoptosis in response to the antineoplastic drug cisplatin.


The laminin-like protein merosin was purified from human placenta in intact form and as pepsin fragments and compared to laminin in heparin affinity chromatography and cell binding assays. Intact merosin and a small fragment of merosin comprising the last two repeats of the heavy chain g domain bind to heparin. Intact merosin and large pepsin fragments of merosin, but not the small C-terminal fragment, mediate the attachment and spreading of several types of cells and promote neurite outgrowth from neuronal cells similar to laminin and its corresponding fragments. Cells with various integrin-type receptors for laminin attached equally well to merosin and laminin, suggesting that several of the known laminin binding receptors also bind to merosin. Antibodies to the [beta]1 subunit of integrins inhibited neurite outgrowth on merosin as well as on laminin, confirming the involvement of integrin-mediated interaction of cells with both merosin and laminin. Schwannoma cells, which have previously been shown to produce a laminin-like, neurite-promoting factor, synthesize merosin in vivo and in vitro as shown by protein and mRNA analysis. The results suggest that merosin, which is the more abundant basement membrane protein in the laminin family, has properties very similar to laminin despite differences in the structure of the heavy chain. Furthermore, merosin may be identical to or a component of the neurite-promoting factors previously reported from heart, muscle, and Schwann cells.

line GD25 to four different laminin variants. The cells were shown to produce dystroglycan, which based on affinity chromatography bound to laminin-1, -2/4, and -10/11, but not to laminin-5. The cells also expressed the integrin [alpha]6A[beta]4A variant. GD25 [beta]1 integrin-null cells are known to bind poorly to laminin-1, but we demonstrate here that these cells bind avidly to laminin-2/4, -5, and -10/11. The initial binding at 20 min to each of these laminins could be inhibited by an integrin [alpha]6 antibody, but not by a dystroglycan antibody. Hence, integrin [alpha]6A[beta]4A of GD25 cells was identified as a major receptor for initial GD25 cell adhesion to three out of four tested laminin isoforms. Remarkably, cell adhesion to laminin-5 failed to promote cell spreading, proliferation, and extracellular signal-regulated kinase (ERK) activation, whereas all these responses occurred in response to adhesion to laminin-2/4 or -10/11. The data establish GD25 cells as useful tools to define the role integrin [alpha]6A[beta]4A and suggest that laminin isoforms have distinctly different capacities to promote cell adhesion and signaling via integrin [alpha]6A[beta]4A.


The critical factors in the regulation of telomere length are not yet clearly defined. Telomerase is a key player in telomere elongation, although previous studies have shown that telomeres are differentially elongated after telomerase reconstitution. Moreover, a clear relation between the level of telomerase activity and telomere length was not observed. To investigate which factors are critical in telomere length regulation, we generated 24 telomerase-reconstituted primary human fibroblast clones. In these clones, in vitro telomerase activity level is clearly related to telomere length. High levels of telomerase activity are associated with longer telomeres and better telomere maintenance over time. The correlation coefficient, however, indicates that the level of telomerase activity is not the only factor in the regulation of telomere length. Clearly, factors that are not measured in an in vitro telomerase activity assay are involved in telomere length regulation in vivo. To investigate which telomerase components are critical in regulating telomerase activity levels, we studied expression levels of hTERT mRNA and hTR. Expression is highly variable between individual clones, but not related to the level of telomerase activity or telomere length. Our results indicate that expression levels of hTERT mRNA and hTR do not regulate the activity level of the telomerase complex, suggesting posttranscriptional modification of hTERT or the presence of additional proteins that modulate telomerase enzyme activity.


Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]), a ligand-activated transcription factor, forms a heterodimer with retinoid X receptor [alpha] (RXR[alpha]), and its transcriptional activity is thought to be maximal in the presence of both PPAR[gamma] and RXR[alpha] ligands. Although previous studies suggested that thiazolidinediones (TZDs), known as PPAR[gamma] ligands, inhibit the growth of several types of tumor cells, the precise mechanism still remains obscure. The present study was designed to examine the effects of PPAR[gamma]/RXR[alpha] transcriptional activation on cell growth in cancer cells. We compared
the effects of six types of TZDs (troglitazone, RS-1303, RS-1330, RS-1387, RS-1455, and RS-1456) and 9-cis RA, an RXR[alpha] ligand, on the activation of PPAR[gamma]/RXR[alpha] and the growth inhibition of six types of adenocarcinoma cell lines (MKN45, HT-29, HCT116, HuCCT1, KMP-2, and BxPC3) established from abdominal malignancies. PPAR[gamma] was expressed in all six tumor cell lines and transcriptionally functional in five of the six lines. The stronger PPAR[gamma] activator showed the stronger growth inhibitor in these five cell lines. However, no significant growth inhibitory effect of six types of PPAR[gamma] activators was observed in BxPC3 cells, which showed no significant PPAR[gamma] transactivation by these activators. Simultaneous addition of troglitazone and 9-cis RA enhanced both activation of PPAR[gamma]/RXR[alpha] and growth inhibition in several types of cancer cells. The degree of PPAR[gamma]/RXR[alpha] activation correlated with the extent of growth inhibition ($r > 0.70, P < 0.05$). This growth inhibition was associated with G1 cell cycle arrest and cell differentiation. These findings suggest that activation of the PPAR[gamma]/RXR[alpha] pathway plays an important role in the growth inhibition of tumor cells and that this nuclear hormone receptor may be a possible novel molecular target for treatment of tumors in humans.


http://www.sciencedirect.com/science/article/B6WFC-47GHN5S-5/2/7a942507d30e335e076e86eeccf49cc3

Activation of fatty acid synthase (FAS) expression and fatty acid synthesis is a common event in human breast cancer. Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that regulate genes involved in lipid metabolism, including FAS. SREBP-1c expression is induced in liver and adipose tissue by insulin and by fasting/refeeding and is critical for nutritional regulation of lipogenic gene expression. In contrast, upregulation of fatty acid metabolism during in vitro transformation of human mammary epithelial cells and in breast cancer cells was driven by increased MAP kinase and PI 3-kinase signaling, which increased SREBP-1 levels. SREBP-1a was more abundant than SREBP-1c in many proliferative tissues and cultured cells and was thus a candidate to regulate lipogenesis for support of membrane synthesis during cell growth. We now show that SREBP-1c and FAS mRNA were both increased by H-ras transformation of MCF-10a breast epithelial cells and were both reduced by exposure of MCF-7 breast cancer cells to the MAP kinase inhibitor, PD98059, or the PI 3-kinase inhibitor, wortmannin, while SREBP-1a and SREBP-2 showed less variation. Similarly, the mRNA levels for FAS and SREBP-1c in a panel of primary human breast cancer samples showed much greater increases than did those for SREBP-1a and SREBP-2 and were significantly correlated with each other, suggesting coordinate regulation of SREBP-1c and FAS in clinical breast cancer. We conclude that regulation of FAS expression in breast cancer is achieved through modulation of SREBP-1c, similar to the regulation in liver and adipose tissue, although the upstream regulation of lipogenesis differs in these tissues.

Experimental Eye Research (6)

Myopia is a condition in which the eye is too long for the focal length of cornea and lens. Analysis of the messengers that are released by the retina to control axial eye growth in the animal model of the chicken revealed that glucagon-immunoreactive amacrine cells are involved in the retinal image processing that controls the growth of the sclera. It was found that the amount of retinal glucagon mRNA increased during treatment with positive lenses and pharmacological studies supported the idea that glucagon may act as a stop signal for eye growth. Glucagon exerts its regulatory effects by binding to a single type of glucagon receptor. In this study, we have sequenced the chicken glucagon receptor and compared its DNA and amino acid sequence with the human and mouse homologues. After sequencing about 80% of the receptor, we found a homology between 79.4 and 75.6% on cDNA level. At the protein level, about 73% of the amino acids were identical. Moreover, the cellular localization and regulation of the glucagon receptor in the chick retina was studied. In situ hybridization studies showed that many cells in the ganglion cell layer and inner nuclear layer, and some cells in the outer nuclear layer, express the receptor mRNA. Injection of the glucagon agonist Lys17,18,Glu21-glucagon induced a down-regulation of glucagon receptor mRNA content. Since the mouse would be an attractive mammalian model to study the biochemical and genetic basis of myopia, and because recent studies have demonstrated that form deprivation myopia can be induced, the expression of preproglucagon and glucagon receptor genes were also studied in the mouse retina and were found to be expressed.


Ocular neovascularisation is the leading cause of blindness in developed countries and the most potent angiogenic factor associated with neovascularisation is vascular endothelial growth factor (VEGF). We have previously described a sense oligonucleotide (ODN-1) that possesses anti-human and rat VEGF activity. This paper describes the synthesis of lipid-lysine dendrimers and their subsequent ability to deliver ODN-1 to its target and mediate a reduction in VEGF concentration both in vitro and in vivo. Positively charged dendrimers were used to deliver ODN-1 into the nucleus of cultured D407 cells. The effects on VEGF mRNA transcription and protein expression were analysed using RT-PCR and ELISA, respectively. The most effective dendrimers in vitro were further investigated in vivo using an animal model of choroidal neovascularisation (CNV). All dendrimer/ODN-1 complexes mediated in a significant reduction in VEGF expression during an initial 24 hr period (40-60%). Several complexes maintained this level of VEGF reduction during a subsequent, second 24 hr period, which indicated protection of ODN-1 from the effects of endogenous nucleases. In addition, the transfection efficiency of dendrimers that possessed 8 positive charges (x=81[middle dot]51%) was significantly better (P=0[middle dot]0036) than those that possessed 4 positive charges (x=56[middle dot]8%). RT-PCR revealed a correlation between levels of VEGF protein mRNA. These results indicated that the most effective structural combination was three branched chains of intermediate length with 8 positive charges such as that found for dendrimer 4. Dendrimer 4 and 7/ODN-1 complexes were subsequently chosen for in vivo analysis. Fluorescein angiography demonstrated that both dendrimers significantly (P<0[middle dot]0001) reduced the severity of laser mediated CNV for up to two months post-injection. This study demonstrated that lipophilic, charged dendrimer mediated delivery of ODN-1 resulted in the down-regulation of in vitro VEGF expression. In addition, in vivo delivery of ODN-1 by two of the dendrimers resulted in significant inhibition of CNV in an inducible rat model. Time course studies showed that the dendrimer/ODN-1
complexes remained active for up to two months indicating the dendrimer compounds provided protection against the effects of nucleases.


http://www.sciencedirect.com/science/article/B6WFD-49N0DVH-1/2/3205ab62a89cfca4feb6c75eeab11ee3

Endotoxin-induced uveitis (EIU) is an animal model of acute ocular inflammation. Cytokines, chemokines, and nitric oxide (NO) have been reported to play important roles. We have determined whether heme oxygenase (HO)-1, a heat shock protein, can suppress EIU. EIU was induced by a footpad injection of lipopolysaccharide (LPS) in male Lewis rats. Hemin, an inducer of HO-1, was injected intraperitoneally 1 hr prior to the LPS injection. HO-1 and HO-2 expression in the iris-ciliary body (ICB) was studied by real time PCR and Western blot analysis. The number of infiltrating cells and the protein concentration in the aqueous humor (AqH) were evaluated by microscopy and by protein assay. The expression of inducible nitric oxide synthase (iNOS), interleukin (IL)-6, tumor necrosis factor (TNF)-[alpha], and IL-1[beta] mRNA was determined by real time PCR. The concentration of nitrate plus nitrite, and levels of IL-6 and TNF-[alpha] in the AqH were also evaluated by Griess reagents and by enzyme-linked immunosorbent assay, respectively. The expression of HO-1 mRNA and protein, induced by LPS, was enhanced significantly by pre-injection of hemin (PPP<0.001). Hemin is effective in inducing HO-1 and in reducing the ocular inflammation induced by LPS probably by down-regulating NO and pro-inflammatory cytokine expression.


http://www.sciencedirect.com/science/article/B6WFD-47YH4V8-1/2/dca4777e5bb8c0fbc4518e4befeaba

Purpose. Previous studies in our laboratory have shown that 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE), a product of 12-lipoxygenase (12-LOX) activity, is the predominant metabolite formed in rabbit corneas after injury. The present study was undertaken to investigate the effects of epidermal growth factor (EGF), hepatocyte growth factor (HGF), and keratinocyte growth factor (KGF) on 12-LOX expression and activity. We also investigated whether 12(S)-HETE mediated the growth factor-induced proliferation of corneal epithelial cells. Methods. Rabbit corneas were stimulated with EGF, HGF, and KGF (10 ng ml-1) for different times. 12-LOX activity was assayed by incubating corneal microsomal preparations with radiolabeled arachidonic acid (AA) as substrate. For inhibitor studies, the microsomes were pretreated with 12-LOX-specific inhibitors baicalein (BC) or cinnamyl 3,4-dihydroxy-(alpha)-cyanocinnamate (CDC). Lipid extracts were injected onto an Ultramex 5 [mu]m C18 column and radioactivity was monitored online by a Radiomatic Flo-One Beta detector. Stereorechemical analysis of 12-HETE product was determined by chiral-phase HPLC. To evaluate the effects of growth factors on 12-LOX mRNA expression, mRNA was extracted at several time points (12, 24, 36, 48 hr) and subjected to real-time PCR. For 12-LOX protein expression, microsomal preparations from 24- and 48-hr incubations were analyzed by Western blot. In cell-proliferation studies, epithelial cells treated with EGF, HGF, or KGF for 24, 48, and 72 hr were measured with a CyQUANT cell-proliferation assay kit. To determine the role of growth factor-induced 12(S)-HETE synthesis on corneal epithelial cell proliferation, cells were pretreated with 12-LOX-specific inhibitors BC or CDC prior to growth-factor supplementation. Results. Stimulation with EGF, HGF, or KGF for 12 hr induced 12-LOX mRNA expression in rabbit corneal epithelial cells. This gene induction was followed by an
increase in protein expression at 24 and 48 hr and a marked increase in 12(S)-HETE synthesis when compared to untreated controls. At 24-hr incubations, KGF showed a greater capacity than did EGF and HGF to stimulate microsomal 12-LOX activity, while at 48 hr 12(S)-HETE synthesis was significantly greater in EGF-treated cells as compared to that of HGF- and KGF-treated cells. Pretreatment with 12-LOX inhibitors blocked the growth factor-induced increase in 12(S)-HETE synthesis. Stimulation with growth factors or 12(S)-HETE for 24, 48, and 72 hr produced a significant increase in corneal epithelial proliferation, which was partially inhibited by pretreatment of cells with 12-LOX-specific inhibitors. Conclusion. These findings suggest that EGF, HGF, and KGF stimulate 12(S)-HETE production in rabbit corneal epithelial cells through gene induction of 12-LOX. Furthermore, 12(S)-HETE may play a role in regulating epithelial cell proliferation and the rate of corneal re-epithelialization following an injury.


http://www.sciencedirect.com/science/article/B6WFD-4D3B124-1/2/c6d3184a75fe087d7001bac8d59c7180

Neovascularization stimulated by IGF-1 mediated induction of vascular endothelial growth factor (VEGF) is one of the leading causes of blindness in humans. It plays a central role in the pathogenesis of proliferative diabetic retinopathy (DR), neovascular glaucoma, exudative age-related macular degeneration (AMD) and retinopathy of prematurity. Neovascularization is a multi-step process that involves complex interactions of a variety of mitogenic factors such as VEGF and IGF-I which are produced locally in the human eye by a variety of cells including retinal pigment epithelial (RPE) cells, retinal capillary pericytes, endothelial cells, Mueller cells and ganglion cells. We hypothesized that somatostatin would inhibit the IGF-1 signal transduction pathway in RPE cells, resulting in decreased VEGF production. We have observed expression of somatostatin receptor protein in retinal pigment epithelial (RPE) cells of the human eye using immunohistochemistry and have confirmed expression of somatostatin receptors in cultured human RPE cells using reverse transcriptase-PCR. IGF-1 induced a dose dependent increase in IGF-1R phosphorylation and in VEGF mRNA levels in cultured human RPE cells. Somatostatin and octreotide, a somatostatin analogue, inhibited IGF-1 receptor (IGF-1R) phosphorylation and decreased VEGF production. Both IGF-1R phosphorylation and accumulation of VEGF mRNA were inhibited by physiological levels of somatostatin and octreotide (1 nM). These results demonstrate somatostatin and octreotide mediated attenuation of both IGF-1R signal transduction and VEGF mRNA accumulation via somatostatin receptor type 2 (sst2). Furthermore, these data suggest a rationale for the use of octreotide as a prophylactic and therapeutic option in disease states that cause ocular neovascularization.


http://www.sciencedirect.com/science/article/B6WFD-47K2MTC-2/2/819cd9dfbe246f0c9c7e75f31f577f1

The aim of this study was to elucidate the expression of chemokines, their role and regulation in bacterial corneal infection using three bacterial strains (Pseudomonas. aeruginosa- invasive, cytotoxic and contact lens induced acute red eye strains) which have been shown to produce three distinct patterns of corneal disease in the mouse. The predominant chemokine expressed in response to all three strains was MIP-2. Prolonged expression of high levels of MIP-2 was associated with increased severity of corneal inflammation. Significantly reduced disease severity upon administration of anti-MIP-2 antibodies suggested that MIP-2 may play an important role in
the pathogenesis of Pseudomonas keratitis at least in part by being a major chemoattractant for polymorphonuclear leukocytes (PMN) recruitment. Interestingly, the numbers of bacteria in eyes with neutralized MIP-2 activity did not decrease even though the severity of the disease was decreased. This implies PMNs as the major destructive factor in microbial keratitis. Further, neutralization of IL-1[beta] activity alone using monoclonal antibodies resulted in significant reduction of both MIP-2 and KC activity indicating that chemokine levels were regulated by IL-1[beta]. These studies demonstrate that the regulation of MIP-2 activity may be beneficial in reducing corneal damage during microbial keratitis in rodents and perhaps that regulation of the human homologue of MIP-2, IL-8, may be useful for controlling keratitis in humans.

**Experimental Gerontology**  (10)


http://www.sciencedirect.com/science/article/B6T6J-48HXT1W-3/2/0a720d1a61ba4af09f3dbb3c26d951c

Adenosine (Ado), a naturally occurring autacoid, exerts cardioprotective effects against myocardial ischemia and reperfusion injury, through activation of its receptors type 1 (A1) and 2A (A2A). Since ageing involves a complex change in these effects, we evaluated A1 and A2A gene expression in left (LV) and right ventricle (RV) from 2-, 5-, 12-, and 21-month-old Sprague-Dawley rats. LV end-diastolic (EDD) and end-systolic (ESD) internal dimensions (mm) and LV fractional shortening (FS, %) were measured by M-mode echocardiography. Senescence was associated with a reduction in FS (42+/−1, 38+/−2, 39+/−2 and 35+/−2, in 2-, 5-, 12- and 21-month-old rats; p<0.002). Ado A1 mRNA levels were highest in 12 and 21-month-old animals in both ventricles (LV: p<0.001). By contrast, Ado A2A gene expression was lower in the aged LV (p<0.001). These modifications of Ado receptor gene expression and especially the increase in A1 receptor mRNA may partially explain the stronger antiadrenergic effects of Ado in the senescent heart.


http://www.sciencedirect.com/science/article/B6T6J-3V4XPND-7/2/cc46f1b220b2302ddd9f97548ef169e

In this study, we have used the mRNA differential display technique to investigate the changes in gene expression that occur in the process of cellular aging. A number of cDNAs whose corresponding mRNAs are either increasingly or decreasingly expressed in senescent cells were thereby isolated. Through DNA sequencing, one of these differentially displayed mRNAs was identified as mitochondrial ADP/ATP translocase. The altered expression of ADP/ATP translocase in different stages of senescent fibroblasts was further confirmed by Northern blots and semiquantitative RT-PCR. Our results demonstrate that expression of ADP/ATP translocase is progressively decreased during the process of in vitro cellular senescence. Further analyses with MTT assays indicate that the decreased expression of ADP/ATP translocase in senescent cells is in parallel with the decline of mitochondrial functions, suggesting that altered expression of this important mitochondrial enzyme might play an active role in the process of cellular


Since there is still debate about the ability of the aged liver to regenerate, we compared some aspects of this response in young, adult and old rodents. 2, 6, 12 and 19-month-old rats were intraperitoneally injected with CCl4 (3 mg/kg) or left untreated (CT) and killed either 2 h (group A) or 24 h (group B) after intoxication. Liver injury was checked histologically and by assaying transaminases. mRNA levels of albumin (Alb), c-fos, c-myc, hepatocyte growth factor (HGF), transforming growth factor (TGF)-[alpha] and TGF-[beta]1 were also analyzed. Heat shock protein (HSP)70 gene expression was evaluated, and liver GSH content. Transaminases and histology show more damage in aged rats. Alb mRNA was reduced starting at 12 months in group A and at all ages in group B; c-fos and c-myc mRNAs reached the highest levels in 6-month-old rats and the lowest in those aged 12 and 19 months of group A. In group B, c-fos was detectable only in 6-month animals, but c-myc at all ages. HGF, TGF-[alpha] and TGF-[beta]1 mRNAs were up-regulated in treated rats, but to a lesser extent in the aged. HSP70 mRNA, absent in CT, was significantly increased at the age of 6 months, undetectable in the oldest rats in group A; in group B it was only visible in 6-month animals. GSH content was reduced with aging. In conclusion, during aging the liver regenerative machinery is preserved but its activation is reduced and delayed.


http://www.sciencedirect.com/science/article/B6T6J-436W05C-K/2/bc62e4c5be71c1d37287a0aa72482210

Neurons express proteins of the classical complement pathway, including C9. Both the mRNA and protein levels for C9 are sharply upregulated in brain areas affected by Alzheimer's disease (AD). Since little is known about the signals that are responsible for this upregulation, we evaluated in human SH-SY5Y neuroblastoma cells the factors which stimulate C9 production. Interferon-[gamma], phorbol myristate acetate and interleukin-6 all stimulated C9 mRNA expression but the inflammatory cytokines tumor necrosis factor-[alpha], interleukin-1[beta], as well as the anaphylatoxin C5a and the bacterial lipopolysaccharide, were ineffective. Immunohistochemical analysis of postmortem human brains for C9 protein demonstrated its presence in many cortical pyramidal neurons in AD, Down's syndrome, the parkinsonism dementia complex of Guam and pallido-ponto-nigral degeneration, as well as in thalamic neurons of progressive supranuclear palsy and ballooned neurons of Pick's disease. Since C9 is required for the membrane attack complex of complement to become functional, interfering with signaling pathways that stimulate its production could offer new therapeutic strategies for treating various neurodegenerative disorders.

Marzban, G., J. Grillari, et al. (2002). "Age-related alterations in the protein expression profile of
The aim of our study was to monitor the protein expression profile in pituitary glands of healthy C57BL/6J mice during aging. Pituitary glands of 4-week old (immature), 3-month old (mature), and >25-month old mice were analysed by proteomic tools such as two-dimensional electrophoresis and N-terminal micro-sequencing. A change was detected in the expression of growth hormone after sexual maturation. Our particular interest, however, was directed against up-regulated proteins in the old pituitary glands, which are proposed to be involved in the process of neuroendocrine aging. Among these proteins, the expression of glutathione-S-transferase (GST) and apolipoprotein A-1 were increased in old pituitaries. Furthermore, ubiquitin carboxyl-terminal hydrolase (UCH-L1) was significantly up-regulated in senescent C57BL/6J mouse pituitaries. Since only the rat homologue was known, we isolated and analysed the mouse UCH-L1 sequence. Since GST is involved in antioxidative defence and UCH-L1 is part of the ubiquitin/proteasome system, which is responsible for the removal of damaged proteins, these results suggest increased oxidative burden and an increased activity of the ubiquitin system.

Aging alters the vascular response to extracellular nucleotides. However, the molecular mechanisms that underlie the effect of aging remain unclear. We investigated the mRNA expression of P2X1, P2Y1, P2Y2 subtypes of the nucleotide receptors (P2) in the basilar artery, aorta and carotid artery from male Sprague-Dawley rats, 2-months and 19-months old. In the basilar arteries of 19-month old rats, as compared to the 2-month old rats, the P2X1 receptor transcripts were reduced and the P2Y1 and P2Y2 receptor mRNA was increased. In the aorta and carotid arteries, P2Y1 receptor mRNA was decreased in the 19-month old rats when compared to the 2-month old rats. There were no marked changes of P2X1 and P2Y2 receptor mRNA between the two age groups in the aorta or carotid artery. In endothelial cells, P2Y1 and P2Y2 receptor mRNA was reduced with age. We concluded that, down-regulation of P2X1 and up-regulation of P2Y1, P2Y2 receptor mRNA in smooth muscle cells and down-regulation of P2Y1 and P2Y2 receptor mRNA on vascular endothelial cells might underlie the changes of cerebral vascular tone in aging.

Mice in which the p66SHC specific region of the SHC gene is deleted live 30% longer without apparent disease. These mice have lower levels of oxidative stress and apoptosis, both of which have been linked to old age survival in man. This makes SHC1 an important candidate gene for longevity in humans. We found no variations in the p66 specific region of the SHC1 gene in 30 young and 30 extreme long-lived subjects. Thus in man, no common sequence variations occur in p66 specific region of the SHC1 gene. In two independent cohorts of respectively 730 and 563
subjects aged 85 and over, we tested the only known non-synonymous polymorphism, Met410Val, for association with longevity using a prospective follow-up design. In the first cohort, we found increasing valine allele frequency in three strata of increasing age at death (2.8-5.2%). Moreover, compared to Met/Met carriers, mortality rate was a factor of 0.71 (95% CI 0.45-1.13) reduced for Met/Val carriers in the combined cohorts, with similar risk estimates in both cohorts. Low valine allele frequency resulted, however, in low power to detect statistical significance. These data suggest that an association between the Met410Val polymorphism and longevity in humans may exist.


Repair of mismatches in mammalian cell DNA is mediated by a complex of proteins that constitute the so-called mismatch repair system (MMR), the main post-replicative pathway for the correction of replication errors. Loss of MMR (as exemplified by germline mutations in some MMR genes, leading to hereditary non-polyposis colorectal cancer) results in increased mutation rates at both coding sequences and in non-coding regions such as microsatellites. In order to evaluate possible functional alterations of this repair system during ageing that could affect immune system efficiency, we studied microsatellite instability at five different loci interspersed in the genome (CD4, VWA31, Tpox, Fes/FPS and p53) in total DNA from T lymphocyte clones derived from hematopoietic stem cells, or peripheral T cells of young or elderly subjects. In addition, these clones had been maintained for different periods in vitro to represent a culture model of ageing. We observed increasing instability accumulating with increasing passages in culture, particularly in CD34+ cell-derived clones, but no clear donor age relationship.


The effect of aging on gingival fibroblasts in response to bacterial infection was studied. Rat gingival fibroblast (rGF) cells were cultured from gingival tissue removed from young (6 weeks old) and old (20 months old) rats. Both types of rGF cells were challenged with lipopolysaccharide (LPS) from the periodontal pathogen Campylobacter rectus. The levels of prostaglandin E2 (PGE2) and interleukin 1[beta] (IL-1[beta]) released into the cultured medium were measured by a specific radioimmunoassay. LPS stimulated PGE2 and IL-1[beta] production in a dose- and time-dependent manner in rGF cells from both young and old rats was seen. Production of PGE2 and IL-1[beta] by rGF cells from the old rats was higher than those from the young in response to LPS. This greater ability from the older rGF cells to produce PGE2 and IL-1[beta] was due to higher mRNA levels of cyclooxygenase 2 and IL-1[beta], respectively. In contrast, cyclooxygenase-1 and IL-1[beta] converting enzyme gene mRNA levels remained unchanged. Because LPS-stimulated PGE2 and IL-1[beta] production was enhanced by in vivo cellular aging, aging of GF may affect the severity of inflammation and bone resorption by producing a large amount of PGE2 and IL-1[beta] in response to bacterial infection.

http://www.sciencedirect.com/science/article/B6T6J-4CXKN7M-1/2/2f29fe81c4ba6f464e8de42b0f1672e5

Aging is associated with an impaired capacity of the immune system to respond properly to danger signals, such as infection and cancer. Here, we provide evidence that an impaired innate immune response, as measured by a low production capacity of pro- and anti-inflammatory cytokines upon ex vivo standardized danger signalling with bacterial LPS, is predictive for frailty in elderly people: participants who at age 85-year produced low levels of LPS-induced IL-1[beta], IL-6, TNF-[alpha] and IL-1Ra and IL-10, were found to have a more than 2-fold elevated overall mortality risk, independent of chronic illnesses (relative risk is 2.21, 95% confidence interval 1.27-3.82, P=0.005), compared to peers with a higher production of any of the pro- and/or anti-inflammatory cytokines. A significant genetic association with the IL-10 promoter gene was found, indicating that people who are genetically predisposed low cytokine producers are at a higher risk of losing the capacity to respond properly to danger signals with aging. We conclude that a malfunctioning innate immune response predicts frailty in old age and is under specific (immuno-) genetic control.

Experimental Hematology (29)


http://www.sciencedirect.com/science/article/B6VP8-49V4XXY-C/2/a6de57b70ed0a54af24386f2e08045b2

Objective

The gene BAALC (Brain And Acute Leukemia, Cytoplasmic), a novel molecular marker involved in leukemia, is highly expressed in a subset of patients with acute leukemia and predictive of clinical outcome in patients with acute myeloid leukemia and normal karyotype. The role of BAALC in hematopoiesis and leukemogenesis is unknown. Material and methods

We used real-time RT-PCR to show that BAALC is strongly expressed in CD34+ cells from the bone marrow and blood and only weakly expressed in total normal bone marrow and blood cells. Results

Expression analyses of FACSorted cells revealed high BAALC transcript levels in CD34+ bone marrow cells including CD34+/CD38-, CD34+/CD33+, as well as CD34+/CD19+/CD10+, CD34+/CD7+, and CD34+/CD71+/CD45- cell fractions. Expression was significantly lower in all CD34- fractions. In vitro differentiation of CD34+ bone marrow cells showed downregulation of BAALC and CD34 transcripts as early as day 4 in suspension cultures supplemented with lineage-specific cytokines (G-CSF, M-CSF, or EPO). In cultures with only lineage-unspecific cytokines (IL-3, SCF, GM-CSF), BAALC transcripts persisted up to day 20, while CD34 transcripts disappeared earlier. These observations suggest that expression of BAALC is stage specific. Conclusions

BAALC expression is restricted to progenitor cells, and downregulation of BAALC occurs with cell differentiation. We postulate that BAALC represents a novel marker of an early progenitor cell common to the myeloid, lymphoid, and erythroid pathways.

http://www.sciencedirect.com/science/article/B6VP8-40MT25M-D/2/d4be8ac50cedbb3af9fa3af503e3bb1b

Objective
Bone marrow stromal cells (BMSC) are an attractive target for novel strategies in the gene/cell therapy of hematologic and skeletal pathologies, involving BMSC in vitro expansion/transfection and reinfusion. We investigated the effects of in vitro expansion on BMSC pluripotentiality, proliferative ability, and bone-forming efficiency in vivo.

Methods
BMSC from three marrow donors were cultured to determine their growth kinetics. At each passage, their differentiation potential was verified by culture in inductive media and staining with alizarin red, alcian blue, or Sudan black, and by immunostaining for osteocalcin or collagen II. First passage cells were compared to fresh marrow for their bone-forming efficiency in vivo. Stromal cell clones were isolated from five donors and characterized for their multidifferentiation ability. The lifespan and differentiation kinetics of five of these clones were determined.

Results
After the first passage, BMSC had a markedly diminish proliferation rate and gradually lost their multiple differentiation potential. Their bone-forming efficiency in vivo was reduced by about 36 times at first confluence as compared to fresh bone marrow. Experiments on the clones yielded comparable results.

Conclusions
Culture expansion causes BMSC to gradually lose their early progenitor properties. Both the duration and the conditions of culture could be crucial to successful clinical use of these cells and must be considered when designing novel therapeutic strategies involving stromal mesenchymal progenitor manipulation and reinfusion.


http://www.sciencedirect.com/science/article/B6VP8-49V4XXY-F/2/8def88a89621ac94b93e6dabf9639bb3

Objective
The expression of an MDS1-EVI1-like-1 (MEL1) gene is reported in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with translocation t(1;3)(p36;q21). MEL1 (at chromosome band 1p36.3) is thought to be transcriptionally activated as a result of juxtaposition to the RPN1 gene at 3q21. It is not known whether MEL1 expression is restricted to cases with this particular translocation.

Materials and Methods
Using real-time polymerase chain reaction, we measured MEL1 expression levels, normal bone marrow, and distinct blood cell fractions in 162 de novo AML patients. We also investigated the existence of an EVI1-like gene (EL1) by applying the same method. The existence of these transcripts was confirmed by Northern blot analysis.

Results
MEL1 expression was detected in 87% (141/162) of de novo AML patients. The EL1 transcript also was detected in the majority of the patients. EL1 expression levels highly correlated with MEL1 expression levels in AML cases. Variable MEL1/EL1 expression levels were observed. However, all the patients with favorable-risk karyotypes, i.e., with t(15;17), t(8;21), or inv(16), showed low MEL1/EL1 expression levels. Expression analysis of MEL1/EL1 compared with MDS1-EVI1/EVI1 in distinct normal marrow or blood cell fractions revealed that 1) all four gene products are expressed in CD34+ progenitor cell fractions; 2) both MEL1 and EVI1 are turned down in neutrophils and monocytes/macrophages; while 3) MDS1-EVI1 and EL1 remain expressed in mature blood cell fractions.

Conclusion
Our data suggest that simultaneous low MEL1/EL1 expression in AML is abnormal and that favorable disease is highly associated with this abnormal phenotype.
ObjectiveThe aim of the present study was to investigate the capacity of normal immune blood cells from non-Hodgkin's lymphoma patients to produce tumor necrosis factor (TNF) after lipopolysaccharide (LPS) stimulation and the influence of the TNF (-308) polymorphism in this production.

Materials and methodsA whole peripheral blood cell assay was utilized following LPS stimulation. At selected incubation times, supernatants were harvested for protein dosage, while mRNA was extracted and reverse-transcribed. The amount of TNF mRNA was quantified using real-time quantitative polymerase chain reaction (PCR) and genomic DNA was typed for TNF (-308) polymorphism.

ResultsUpon LPS stimulation, TNF-secreted protein was slightly but not significantly increased in lymphoma patients when compared to controls. In contrast, the relative TNF mRNA amounts were significantly higher in lymphoma patients at 30 minutes (median 27.75 vs 16.00; Mann-Whitney U-test p = 0.02). ConclusionThe LPS-induced TNF mRNA levels are higher in peripheral blood cells (PBC) from lymphoma patients than from controls, while TNF protein secretion is not strikingly different. Altered regulation of TNF mRNA translation or TNF protein secretion may contribute to these observations. Taken together, an increased susceptibility for TNF gene transcription after LPS stimulation was observed in PBC (mainly in monocytes) from lymphoma patients, and especially those carrying the TNF2 allele.


ObjectiveBased on previous animal experiments that suggest the plasticity of peripheral blood-derived, CD34- stem cell lines, the aim of this study was to isolate CD34- stem cell lines from human peripheral blood cells and obtain evidence of their multipotency and plasticity.

Materials and methodsAdherent growing cells were isolated from peripheral blood mononuclear cells from a healthy volunteer donor and different cell clones were established after SV40 large-T-antigen-mediated immortalization. The immunophenotype of the cell lines was investigated by flow cytometry. One particular cell clone, V54/2, was stained with rhodamine 123, and the Rh123low and Rh123high subpopulations were sorted for a reverse transcriptase polymerase chain reaction gene expression survey and distinct differences in morphology and biologic behavior.

ResultsThe peripheral blood-derived and fibroblast-like cell line V54/2 expressed high levels of CD10 and CD105 and showed only a very low level expression of CD34 (CD105+ cell population that transcribed factors such as Myb, Tie-1, and VEGF, there was a small Rh123lowCD34+ subpopulation that transcribed significant levels of several members of the GATA family of transcription factors. The morphology of the Rh123lowCD34+ (also expressing the P-glycoprotein) was different compared to the Rh123highCD34- population. Mesenchymal differentiation into glial fibrillary acidic protein (GFAP)+ glial cells could be shown from the entire CD34-CD105+ cell population.

ConclusionsThe findings provide evidence that it is possible to isolate CD34-CD105+ mesenchymal stem cell lines from human peripheral blood cells that contain a small subpopulation of CD34+ and GATA-transcribing cells. Those cells are potential hematopoietic progenitors and can be recruited from the CD34- stem cell pool. The plasticity of stem cells seems to require essential molecular tools, such as a panel of transcription factors, to respond to the environmental demand within a biologic system.

http://www.sciencedirect.com/science/article/B6VP8-3Y3XR57-5/2/c419fa712a04dda3ac8747fa0495ce0e

Tumor growth is associated with neutrophilia, thrombocytosis, and extramedullar hematopoiesis. The mechanism(s) accounting for these phenomena is unclear, although granulocyte-macrophage colony-stimulating factor (GM-CSF) and/or granulocyte colony-stimulating factor (G-CSF) released by tumor cells have been involved. We studied whether CSF released by Ehrlich tumor (ET) may play a role. A comparative study was performed with two cell variants (ET and ET/0) growing in euthymic, nude, and SCID mice. Extramedullar hematopoiesis was assessed in the spleen by scoring organ enlargement, wheat germ agglutinin ve+ cells, and interleukin 3-dependent granulocyte-macrophage colony-forming unit (GM-CFU). Both cell lines showed the same cytokine profile by reverse transcriptase polymerase chain reaction, including GM-CSF, G-CSF, and macrophage colony-stimulating factor (M-CSF); yet, only ET cells produced detectable colony-stimulating activity in vitro, mainly due to GM-CSF. No differences in tumorigenicity were noted between ET and ET/0 cells inoculated to normal or immunodeficient mice. An increase in extramedullar hematopoiesis, accompanied by neutrophilia and thrombocytosis, was associated with tumor progression irrespective of the cell line. A strong correlation was obtained between the increase in splenic GM-CFU and tumor mass ($r = 0.96, p = 0.0001$) that was independent on the tumor cell line, strain of mice, or stage of tumor development. The results point against CSF released by tumor cells and/or reactive host T cells as the only factors involved in the extramedullar hematopoiesis in this tumor model. The remarkable correlation between splenic GM-CFU and the tumor mass still suggests that a factor(s) of tumor origin may play a critical role.


http://www.sciencedirect.com/science/article/B6VP8-46BMVH2-7/2/5b10a4836269c44bbd984d8ac70c080

ObjectivesShwachman-Diamond syndrome (SDS) is characterized by varying degrees of marrow failure. Retrospective studies suggested a high propensity for malignant myeloid transformation into myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The study's aims were to determine the cellular and molecular characteristics as well as the clinical course of malignant myeloid transformation and clonal marrow disease in patients with SDS. Methods This is a longitudinal prospective study of 14 patients recruited for annual hematological evaluations. Results of baseline and serial hematological assessments for up to 5 years are reported. Results Clonal marrow cytogenetic abnormalities (CMCA) were detected in 4 patients (29%) on first testing or at follow-up. The abnormalities were del(20q) in two patients, i(7q) in one, and combined del(20q) and i(7q) in one. The following tests did not distinguish patients with CMCA from other SDS patients: severity of peripheral cytopenia, fetal hemoglobin levels, percentage of marrow CD34+ cells, colony growth from marrow CD34+ cells, cluster-to-colony ratio, marrow stromal function, percentage of marrow apoptosis cells, and granulocyte colony-stimulating factor receptor expression. RAS and p53 mutation analysis and AML blast colony assays were uniformly negative. No patients showed progression into more advanced stages of MDS or into AML. In one patient, the abnormal clone became undetectable after 2 years of follow-up. Conclusions We conclude that although CMCA in SDS is high, progression into advanced stages of MDS or to overt AML may be slow and difficult to predict. Treatment should be cautious since some abnormal clones can regress.

http://www.sciencedirect.com/science/article/B6VP8-47CWDYT-3/2/8d1a7b201cfd74b0044c3f9966c36eeb

Objective
This study investigated the effect of interleukin-9 (IL-9) on the proliferation and differentiation of human colony-forming unit megakaryocytic progenitor cells (CFU-Meg).

Materials and Methods
Peripheral blood-derived CD34+IL-6R- cells were sorted and cultured in the presence of IL-9, erythropoietin (Epo), stem cell factor (SCF), and thrombopoietin (TPO) alone or in combination. The number of pure and mixed megakaryocyte colonies, the size of pure megakaryocyte colonies, the ploidy distribution of megakaryocytes, and proplatelet formation were investigated.

Results
Apart from TPO, no single factor could support CFU-Meg-derived colony formation, but each two-factor combination among IL-9, Epo, and SCF supported a few CFU-Meg colonies. Interestingly, the combination of Epo+SCF+IL-9 induced four to six times as many CFU-Meg colonies as any of the two-factor combinations. Neutralizing monoclonal antibodies (mAbs) for IL-9 receptor and c-kit completely abolished this synergistic effect. In contrast, addition of neutralizing anti-c-Mpl or anti-CXCR4 Abs did not influence colony formation, indicating that this synergistic effect was independent of TPO or SDF-1. Moreover, the endogenous production of TPO by cultured CD34+IL-6R- cells in the presence of Epo+SCF+IL-9 was ruled out by reverse transcriptase polymerase chain reaction for TPO mRNA. Interestingly, the combination of TPO, Epo, SCF, and IL-9 supported the largest number of pure and mixed megakaryocyte colonies, suggesting that this combination of cytokines might recruit primitive megakaryocytic as well as multipotential progenitors. This combination also potently enhanced proplatelet formation compared with TPO alone or a combination of Epo, SCF, and IL-9.

Conclusion
This study demonstrated for the first time that human IL-9 can potentiate human megakaryocytopoiesis in the presence of Epo and/or SCF.


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Objective
Expression of the cytokine receptor CD30 is a typical feature of anaplastic large cell lymphomas (ALCL). CD30-induced effects have a great impact on cell activation and viability.

Materials and methods
Using Karpas 299 cells, we performed differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) to identify novel genes involved in CD30 signaling in ALCL. Activation of CD30 was induced by treatment with immobilized anti-CD30 antibody. RNA and protein expression were confirmed in different cell lines by Northern and Western blot analysis. Fluorescence-activated cell sorting (FACS) analysis was applied to examine cell viability. Nuclear factor [kappa]B (NF[kappa]B) pathways were blocked using a specific inhibitor.

Results
We found strongly enhanced expression of the cellular inhibitor of apoptosis cIAP1 and cIAP2 in Karpas 299 cells stimulated with anti-CD30. Furthermore, we showed that CD30-regulated expression of cIAP1 and cIAP2 was mediated by NF[kappa]B. Induction of NF[kappa]B, cIAP1, and cIAP2 correlated with partial protection from apoptotic cell death caused by etoposide. Correspondingly, inhibition of the NF[kappa]B pathway not only prevented the prevalent antiapoptotic effects mediated by CD30, but even led to CD30-induced apoptosis. Finally, we found enhanced expression of cIAP1 and cIAP2 in several other ALCL cell lines and the HD-derived cell line HDLM-2 upon CD30 stimulation.

Conclusions
Our results indicate that CD30-mediated protection from apoptosis is a common feature of CD30+ cells. Therefore, CD30-induced signaling may have a significant impact on the clinical outcome of
patients with ALCL.


We have evaluated the in vivo amplification potential of purified murine hematopoietic stem cells, identified as Wheat Germ Agglutinin+ (WGA+), 15-1.1-, Rhodamine 123 Dull (Rho-dull) cells, by serial transplantation into stem cell defective nonmyeloablated W/Wv mice. C57BL Rho-dull cells (250/500 cells/mouse) permanently engrafted nonablated W/Wv mice as defined by the presence of > 95% red and > 20% white donor-derived circulating cells for at least 1.5 years following transplantation. At this time, approximately 61% of Rho-dull cells and all the Rho-bright progenitor and colony forming cells of the engrafted mice were found to be donor-derived by c-Kit genotyping and by their response to stem cell factor (SCF). Retransplantation of 250-1000 Rho-dull cells from primary into secondary W/Wv recipients generated C57BL hematopoiesis in 40%-64% of animals revealing the presence of donor derived hematopoietic stem cells (HSC) in the bone marrow of the primary recipients. One and half years after transplantation, the bone marrow of the secondary engrafted animals contained C57BL Rho-dull cells ([cong] 51% by genotype), which were capable of reconstituting tertiary W/Wv recipients. In this respect, 25% of tertiary mice expressed C57BL hematopoiesis when transplanted with 250-1000 Rho-dull cells purified from secondary W/Wv recipients. On the basis of the number of Rho-dull cells purified from a single mouse, we calculate that approximately 7.3 x 104 Rho-dull cells, which are genotypically and functionally defined as C57BL long-term repopulating stem cells, were generated in the marrow of reconstituted primary W/Wv recipients transplanted 1.5 years earlier with 250-500 C57BL Rho-dull cells. We conclude that murine HSC have extensive amplification capacity in nonmyeloablated animals.


Objective. Genetic alterations, including p53 mutations, have been identified in the stroma of solid tumors and are thought be involved in the induction of tumor growth and metastasis. We tested the hypothesis that somatic molecular alterations in bone marrow stromal cells provide a favorable growth environment for leukemic cells.

Materials and Methods. We established an in vitro model consisting of stroma expressing mutant p53 (Cys135Ser) to study its ability to support growth of cells from a pre-B acute lymphoblastic leukemia (ALL) cell line. Normal and leukemic bone marrow stromal cells were screened for p53 mutations by mutant-specific ELISA, SSCP, and direct sequencing. Secretion of vascular endothelial growth factor (VEGF) was measured by quantitative ELISA.

Results. Transfection of stromal cells with mutant p53 increased synthesis of VEGF and supported the growth of leukemic cells. An ELISA-based assay suggested the occurrence of in vivo p53 alterations in bone marrow stromal cells provide a favorable growth environment for leukemic cells. Materials and Methods. We established an in vitro model consisting of stroma expressing mutant p53 (Cys135Ser) to study its ability to support growth of cells from a pre-B acute lymphoblastic leukemia (ALL) cell line. Normal and leukemic bone marrow stromal cells were screened for p53 mutations by mutant-specific ELISA, SSCP, and direct sequencing. Secretion of vascular endothelial growth factor (VEGF) was measured by quantitative ELISA.

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Results. Transfection of stromal cells with mutant p53 increased synthesis of VEGF and supported the growth of leukemic cells. An ELISA-based assay suggested the occurrence of in vivo p53 alterations in bone marrow stromal cells provide a favorable growth environment for leukemic cells. Material...
one possible pathway by which this process is mediated.


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ObjectiveThe aim of this study was to assess the gene transfer efficiency of an in situ administration protocol for hematopoietic stem/progenitor cells in the rhesus macaque (Macaca mulatta) animal model. Materials and Methods Moloney murine leukemia virus amphotropic vector producer cells (1-2 x 10^8 cells/animal) were transplanted into the femoral bone marrow cavities of six macaques. To determine if the levels of gene transfer could be increased, a second injection at the same dose of producer cells was performed into the iliac crest in three of the six macaques. Results We demonstrated that 0.02-0.1% of peripheral blood mononuclear cells contained the vector transgene for up to 12 months following the initial administration of producer cells. Hematopoietic progenitor cell assays indicated that the neomycin phosphotransferase gene was detected in 10-30% of progenitor cell colonies. A humoral immune response directed toward viral particles was demonstrated in all animals. Additionally, we demonstrated that an increase in the levels of transduced cells, up to 1% of circulating peripheral blood mononuclear cells and granulocytes, contain the transgene following producer cell readministration. Conclusions These data demonstrate the successful in situ gene transfer to hematopoietic stem/progenitor cells and circulating peripheral blood mononuclear cells that persists as long as 12 months postinjection, in the absence of any preconditioning.


http://www.sciencedirect.com/science/article/B6VP8-42RMMV6-1/2/f929de85a406913d002770173ead09de

Objective Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia that is characterized by a deficiency of glycosylphosphatidylinositol-anchored membrane proteins due to phosphatidylinositol glycan-class A (PIG-A) gene abnormalities in various lineages of peripheral blood cells and hematopoietic precursors. The purpose of our study was to clarify the distribution of PIG-A gene abnormalities among various cell lineages during differentiation and maturation in PNH patients. Patients and Methods The expression of CD16b or CD59 in peripheral blood granulocytes or cultured erythroblasts from three Japanese PNH patients was analyzed using flow cytometry. PIG-A gene abnormalities in both cell types, including glycoprophorin A+ bone marrow erythroblasts, were examined using nucleotide sequence analysis. The expression study of PIG-A genes from each patient was also performed using JY-5 cells. Results Flow cytometry revealed that the erythroblasts consisted of negative, intermediate, and positive populations in Cases 1 and 3 and negative and intermediate populations in Case 2. The granulocytes consisted of negative and positive populations in all three cases. DNA sequence analysis indicated that all the PNH cases had two or three types of PIG-A gene abnormalities, and that a predominant clone with an abnormal PIG-A gene was different in granulocytes and erythroblasts from Cases 2 and 3. Expression studies showed that all the mutations from the patients were responsible for the null phenotype. Conclusion PIG-A gene abnormalities result in deficiencies of glycosylphosphatidylinositol-anchored proteins in PNH erythroblasts and granulocytes. The distribution of predominant PNH clones with PIG-A gene abnormalities is often heterogeneous.
between the cell types, suggesting that a clonal selection of PIG-A gene abnormalities occurs independently among various cell lineages during differentiation and maturation.


ObjectiveTo support immune reconstitution after cord blood transplantation, immunotherapy using gene-modified dendritic cells (DCs), the most potent antigen-presenting cells, can be a powerful strategy for preventing infection and recurrence. To investigate the applicability of lentiviral vector-transduced DCs compared to retroviral vectors, we transduced umbilical cord blood (CB) CD34+ cells, then expanded and differentiated them into DCs.Materials and MethodsWe transduced CB CD34+ cells by vesicular stomatitis virus G-protein pseudotyped self-inactivating lentiviral vector or retroviral vectors carrying the enhanced green fluorescent protein gene. The cells were expanded in the stroma-dependent culture system and transferred to the culture condition for developing DCs. The efficiency of transduction and expression of the transgene in severe combined immunodeficiency (SCID) mice-repopulating cells (SRCs) and DCs were compared between lentiviral vector and retroviral vectors. Induced DCs were cocultured with allogeneic or autologous T cells to test the ability to present antigens. Results CB CD34+ cells transduced by lentiviral vector and expanded ex vivo sustained stable transgene expression and multipotentiality by assessing SRCs assay and clonogenic assay of bone marrow cells from the transplanted mice. DCs derived from these cells expressed green fluorescent protein and surface markers CD1a, CD80, and HLA-DR and showed potent allostimulatory activity as well as nontransduced DCs did. On the other hand, we did not detect transgene expression in SRCs and DCs transduced by retroviral vectors. Conclusion Gene-modified DCs derived from ex vivo expanded CB CD34+ cells transduced by lentiviral vector will be useful in future immunotherapy protocols.


Objective Bone marrow stromal cells provide the microenvironment for self-renewal and differentiation of hematopoietic stem/progenitor cells through complex cell-cell interaction. To elucidate the regulatory mechanisms of hematopoiesis by stromal cells, we established a novel stroma-dependent hematopoietic cell line and explored the phenotypic changes regulated by the two stromal cells. Materials and Methods DFC-28 cells clonally established from long-term bone marrow culture of C57BL/6 mice were sustained by coculture on MSS62 cells (mouse spleen stromal cell line). When DFC-28 cells were transferred to TBR31-1 cells (mouse bone marrow stromal cell line), their phenotypic changes were analyzed by flow cytometry and reverse transcriptase polymerase chain reaction. Results DFC-28 cells on MSS62 cells exhibited surface phenotypes of the immature hematopoietic progenitor cells (Lin-AA4.1+c-kit+Sca-1-). By stroma-replacement from MSS62 cells to TBR31-1 cells, DFC-28 cells were differentiated into very early B-lymphoid stage characterized by c-kit down-regulation and induction of BP-1 and B-lymphoid-associated genes (Pax-5, CD19, TdT, Rag-1, and Rag-2). In addition, the differentiation phenotypes reverted to the immature state characterized by c-kit induction and down-regulation of BP-1 and B-lymphoid-associated genes by replacing stroma back to MSS62 from TBR31-1. Interleukin-7 stimulation and conditioned medium of TBR31-1 cells were ineffective in converting
the differentiation phenotypes of DFC-28 cells. 

Conclusion The results demonstrate that the differentiation phenotypes and growth potential of stroma-dependent hematopoietic progenitor cells we established could be reversibly controlled via direct contact with stromal cells in the microenvironment.


http://www.sciencedirect.com/science/article/B6VP8-40MT25M-6/2/43058064f29a23a73c607b3c4820715

Objective To understand regulation of myeloid development, it is necessary to obtain the myeloid progenitor cell lines with self-renewal and differentiation capacities. Because prolonged hematopoiesis occurs with the production of myeloid cells at all stages of differentiation in the Dexter-type long-term bone marrow cultures, we tried to obtain stroma-dependent myeloid progenitor cells starting from the long-term bone marrow culture.

Materials and Methods Murine cobblestone areas generated in long-term bone marrow cultures were serially passaged every 10 days. After 4 months, the resultant hematopoietic cells, designated as DFC, were passaged on a monolayer of established spleen stromal cell line, MSS62. After 10-12 passages of DFC cells on MSS62, several clones were obtained by colony formation on MSS62 cell layer. Among these clones, DFC-a cells could be maintained for a long period by coculturing with the established stromal cell line, MSS62. Results DFC-a cells proliferated by forming cobblestones and contained blast cells, granulocytes, and macrophages. Cell sorting and coculture experiments indicated that the blast type cells exhibiting c-Kit+ Gr-1- Mac-1-, stroma-dependently self-renewed, and spontaneously differentiated toward granulocytes (c-Kit+ Gr-1+ Mac-1+) and macrophages (c-Kitlow/+ Gr-1- Mac-1high). Although most of DFC-a cells expressed c-Kit, SCF-c-Kit interaction was not always necessary for their growth. In the presence of stromal cells, growth and differentiation of DFC-a cells were stimulated by GM-CSF or IL-3. Without stromal cells, DFC-a was transiently expanded by GM-CSF or IL-3 but could not be maintained constantly by these cytokines. Conclusion The present study demonstrated that DFC-a is a novel bipotent myeloid progenitor cell clone as a simple model system of stroma-dependent myeloid development. It may reflect distinct properties for the earliest myeloid progenitor cells in vivo. It is of interest to know what signals are provided by MSS62 stromal cells to maintain the myeloid progenitor cells.


http://www.sciencedirect.com/science/article/B6VP8-40MT25M-5/2/3d9a94dc647befe80e920c18ee00002

Objective We investigated whether gene transfer into hematopoietic cells could be achieved by direct injection of retroviral vector supernatant into the bone marrow space of newborn sheep. Materials and Methods Six sheep (5 weeks old) were injected bilaterally with either 1 mL of G1nBgSvNa8.1 vector supernatant (titer: 1 x 107) in each hip (n = 5) or with 3 mL of the same vector preparation/hip (n = 1). In addition, one 3-month-old sheep was injected unilaterally with 1 mL of the same vector preparation. Blood and marrow of these animals were analyzed for the transgene before injection and at intervals thereafter. Results At 1 week postinjection, an average of 11.6% of the lymphocytes and 25.5% of the granulocytes/monocytes in the marrow, and an average of 0.9% of the lymphocytes and 1.8% of the granulocytes/monocytes in the blood contained and expressed the LacZ gene. The presence/expression of the transgene has persisted for at least 13 months within the blood and bone marrow of these
animals. Conclusions These findings demonstrate that the direct injection of small volumes of high-titer retroviral supernatant into the bone marrow of newborn sheep results in transduction of hematopoietic cells that persists for at least 13 months postinjection.


http://www.sciencedirect.com/science/article/B6VP8-45VCK0N-F/2/a9e4361e430f4bc49c30953801d0c077

Objective Quantitative assessment of gene expression in stem cells is essential for understanding the molecular events underlying normal and malignant hematopoiesis. The aim of the present study was to develop a method for precise quantitation of gene expression in small subsets of highly purified CD34+CD38- stem cell populations. Materials and Methods. Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantitate housekeeping and drug resistance gene expression in cDNA obtained from 300 CD34+CD38- cells without cDNA amplification or nested PCR techniques. Results Validation experiments in cell lines showed efficient, representative and reproducible gene amplification using 300-cell real-time quantitative RT-PCR. Sensitivity was confirmed in dilutional experiments and by detection of the low-copy gene PBGD. GAPDH was found to be a useful reference gene in normal and leukemic CD34+CD38- cells. In contrast, 18S rRNA content varied 100-fold to 1000-fold in these populations. Moreover, expression of 18S rRNA was significantly lower in leukemic CD34+CD38+ cells compared to normal CD34+CD38+ cells (p = 0.002). Expression of MDR-1 (18-fold, p p +CD38- compared to CD34+CD38+ cells. Conclusions Real-time quantitative RT-PCR is a valuable tool for precise quantitation of gene expression in small subsets of hematopoietic cells. Using this method, we showed the inappropriateness of 18S as a reference gene in these progenitors and the down-regulation of drug-resistance-related genes early in hematopoiesis.


http://www.sciencedirect.com/science/article/B6VP8-46YGK0Y-4/2/f2833ab7bb3be386ce705b4da20ea6fd

Objective Chronic myelomonocytic leukemia (CMML) is a heterogeneous disease with no effective treatments or cure. Several factors have been implicated in its pathogenesis. In the current study, we studied the dependence of CMML on granulocyte-macrophage colony-stimulating factor (GM-CSF). Materials and Methods We used in vitro colony assays in methylcellulose where CMML cells were tested in the presence or absence of the specific GM-CSF antagonist E21R. We also developed an in vivo model in which CMML cells were tested for their ability to engraft into immunodeficient mice transgenic for human GM-CSF. Results Bone marrow cells from seven of seven patients with CMML formed spontaneous colonies that were sensitive to E21R treatment, with reduction in colony growth by up to 92%. E21R also inhibited colony formation by CMML patient cells stimulated by exogenously added GM-CSF but not interleukin-3. In vivo experiments we observed engraftment of CMML cells (but not normal cells) in immunodeficient mice transgenic for human GM-CSF. None engrafted in nontransgenic mice. Cell dose escalation showed that the optimal number was 0.5 to 1 x 108 peripheral blood mononuclear cells per mouse, which is equivalent to an infusion of 0.2 to 3.6 x 106 CD34+ cells. Time course experiments showed that maximal engraftment occurred 6 weeks after injection. Conclusions These results demonstrate that in some CMML patients, GM-CSF produced
by either autocrine or paracrine mechanisms is a major growth determinant. The results suggest that therapies directed at blocking this cytokine could control the growth of some CMML patients in vivo.


Objective. Megakaryopoiesis is characterized by two major processes, acquisition of lineage-specific markers and polyploidization. Polyploidy is a result of endomitosis, a process that is characterized by continued DNA replication in the presence of abortive mitosis. Stathmin is a major microtubule-regulatory protein that plays an important role in the regulation of the mitotic spindle. Our previous studies had shown that inhibition of stathmin expression in human leukemia cells results in the assembly of atypical mitotic spindles and abnormal exit from mitosis. We hypothesized that the absence of stathmin expression in megakaryocytes might be important for their abortive mitosis.

Materials and Methods. The experimental models that we used were human K562 and HEL cell lines that can be induced to undergo megakaryocytic differentiation and primary murine megakaryocytes generated by in vitro culture of bone marrow cells. The megakaryocytic phenotype was evaluated by flow cytometry and light microscopy. The DNA content (ploidy) was analyzed by flow cytometry. Stathmin expression was analyzed by Western and Northern blotting and by RT-PCR.

Results. Our studies showed an inverse correlation between the level of ploidy and the level of stathmin expression in megakaryocytic cell lines and in primary cells. More importantly, inhibition of stathmin expression in K562 cells enhanced the propensity of these cells to undergo endomitosis and to become polyploid upon induction of megakaryocytic differentiation. In contrast, inhibition of stathmin expression interfered with the ability of the cells to acquire megakaryocyte-specific markers of differentiation.

Conclusion. Based on these observations, we propose a model of megakaryopoiesis in which stathmin expression is necessary for the proliferation and differentiation of early megakaryoblasts and its suppression in the later stages of megakaryocytic maturation is necessary for polyploidization.


Fabry disease is a lysosomal storage disorder that is due to a deficiency in [alpha]-galactosidase A ([alpha]-gal A). Previously we have shown that a recombinant retrovirus synthesized for the transfer of the human [alpha]-gal A coding sequence was able to engineer enzymatic correction of the hydrolase deficiency in fibroblasts and lymphoblasts from Fabry patients. The corrected cells secreted [alpha]-gal A that was taken up and utilized by uncorrected bystander cells, thus demonstrating metabolic cooperativity. In separate experiments we used transduced murine bone marrow cells and successfully tested and quantitated this phenomenon in vivo. In the present studies, which were designed to bring this therapeutic approach closer to clinical utility, we establish that cells originating from the bone marrow of numerous Fabry patients and normal volunteers can be effectively transduced and that these target cells demonstrate metabolic cooperativity. Both isolated CD34+-enriched cells and long-term bone marrow culture cells, including nonadherent hematopoietic cells and adherent stromal cells, were transduced. The transferred gene generates increased intracellular [alpha]-gal A enzyme activity in these cells.
Further, it causes functional correction of lipid accumulation and provides for long-term [alpha]-gal A secretion. Collectively, these results indicate that a multifaceted gene transfer approach to bone marrow cells may be of therapeutic benefit for patients with Fabry disease.


http://www.sciencedirect.com/science/article/B6VP8-458WBCC-3/2/fe794f219599fb6684ec9900e2bb97c80

ObjectiveCD22 is believed to be restricted to normal and neoplastic B cells. Human basophils were found to express CD22 molecules. Among the antibodies against CD22, Leu14, which recognized the ligand binding domain, reacted to basophils, and B3 and 4KB128, which recognized the amino terminus side and carboxy terminus side of the ligand binding epitope, respectively, did not. To clarify the difference of CD22 antigenicity in human B cells and basophils, we investigated RNA sequence and structures of CD22 molecules.

Materials and MethodsPurified B cells and basophils were obtained from normal human volunteers by using a MACS magnetic cell sorting system and anti-CD19 and anti-Fc[epsilon]R1 antibodies, respectively. RT-PCR and sequencing of CD22 mRNA were performed in the exons 3 to 8. Western blotting analysis of CD22 was also performed.

ResultsThe sequence of CD22 mRNA extracted from the basophils was the same as that of B cells in exons 3 to 8 (epitopes recognized by Leu14, B3, and 4KB128 were translated from exons 4 and 5). Reduced CD22 peptide extracted from the basophils reacted to Leu14 as well as B3 and 4KB128, and the molecular size of the reduced and nonreduced products was 130 kDa as expected.

ConclusionDisulfide bonds and the resulting 3D conformation of the CD22 molecules may have important roles in the difference of antigenicity of CD22[beta] in B cells (CD22[beta]1) and basophils (CD22[beta]2). The difference in molecular structure surrounding the ligand-binding domain of CD22 may imply a specialization of the conformational forms of CD22 according to the ligand isoforms.


http://www.sciencedirect.com/science/article/B6VP8-445G7M5-8/2/f854e9406925c07a5a266df8a7b6896

ObjectiveThe CD36 molecule is expressed in platelets, monocytes, erythroblasts, and other different tissues. The two types of platelet CD36 deficiency, types I and II, are associated with the absence and presence of CD36 on monocytes, respectively. To clarify the involvement of the erythroid lineage in CD36 deficiency, we investigated the phenotype and RNA expression of CD36.

Materials and MethodsCD36 expression was examined in 296 patients with several cardiovascular diseases in our outpatient clinic. There were 12 patients with type I deficiency and 16 with type II CD36 deficiency. A bone marrow sample was examined in five type I and four type II patients. Expression of CD36 mRNA was examined in burst-forming unit-erythroid (BFU-E). The sequences of reverse transcriptase polymerase chain reaction (RT-PCR) products of the CD36 mRNA from monocytes were examined.

ResultsAs expected, CD36 was deficient in erythroblasts from all five patients with type I deficiency. CD36 was present in erythroblasts from three of the four with type II deficiency, suggesting that their abnormality is restricted to platelets (type IIa). CD36 was unexpectedly absent from erythroblasts of a single type II patient (type IIb). CD36-specific mRNA was identified in BFU-E from each of two normals, six type I, and six type II patients, including type IIb. The sequences of RT-PCR products of the CD36 mRNA in a patient with type IIa and another with type IIb showed homozygous wild alleles.

ConclusionThe findings provide evidence for further heterogeneity among CD36-deficient individuals and the existence of
a basic principle mechanism of type II, such as glycosylation abnormality.


http://www.sciencedirect.com/science/article/B6VP8-426H6SJ-F/2/0433a57d8780a51e98c2b694354bf47

Objective The rae28 gene (rae28) is a murine homologue of the Drosophila polyhomeotic gene, which is a member of the Polycomb-group genes. In this study, we examined the role of rae28 in lymphocyte development. Materials and Methods Because homozygous rae28-deficient (rae28-/-) mice died in the perinatal period, we examined lymphocyte development by generating chimeric mice reconstituted with green fluorescence protein-labeled mutant fetal liver cells as well as in vitro culture systems. We further examined RAE28 expression by reverse transcriptase polymerase chain reaction assay in human leukemic cells with B-lineage acute lymphoblastic leukemia (ALL). Results Severe B-cell maturation arrest was observed in rae28-/- between pro- and pre-B lymphocyte stages. B-cell development was also delayed in heterozygous neonates. Furthermore, interleukin-7-dependent colony-forming ability was impaired not only in homozygous lymphocytes but also in heterozygotes. Its human homologue, RAE28, is located on chromosome 12p13, which is frequently associated with chromosomal abnormalities and loss of heterozygosity in patients with hematologic malignancies. To determine whether a link exists between RAE28 and leukemia, we examined RAE28 expression in leukemic cells from pediatric patients with B-lineage ALL. RAE28 expression was not detected in four B-cell precursor ALL cases of a total of 43 examined, although RAE28 is normally expressed constitutively during the process of B-cell maturation as assessed in isolated cell populations. Conclusions rae28 plays an important role in the early B-cell developmental stage in a gene dosage-dependent manner. Furthermore, the human RAE28 locus may provide a candidate gene causing the molecular pathogenesis of childhood B-cell precursor ALL.


http://www.sciencedirect.com/science/article/B6VP8-4F8SXDF-D/2/9835e3c2f757bf6c1bee3c4bc664e5f1

Objective Circadian genes have recently been characterized in many tissues, but not in hematopoietic stem cells. These cells are rare in the bone marrow (BM), which makes it difficult to collect enough cells for detailed molecular analysis in a short period of time without reduced RNA quality. The aim was to improve methodology and reliability of clock gene expression analysis in purified mouse hematopoietic stem cells. Methods Stem cells were highly enriched by high-speed flow cytometric cell sorting of the side population (SP) cells from Hoechst 33342 (Hoechst)-stained mouse BM. Total RNA was isolated from sorted SP and whole BM cells and exposed to DNase treatment. The relative mRNA levels of major clock genes mPer1, mPer2, mBmal1, mCry1, mClock, and mRev-erb [alpha] were measured with real-time quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) and normalized to m36B4, used as a reference gene. The clonogenity of sorted SP cells and whole BM; cells taken before and after sorting, were tested in colony-formation assay. Results Clock gene activity in sorted SP cells showed pronounced relative differences compared with whole BM for mPer1 and mCry1. The high-speed sorting procedure did not influence clock gene expression or cell clonogenity, even when this was performed with a delay period up to 24 hours. Conclusions We demonstrated expression of six clock genes in mouse hematopoietic stem cells. A combination of high-speed flow cytometric sorting and Q-RT-PCR was shown to be useful and reliable for analysis of clock
gene activity in small stem cell fractions.


http://www.sciencedirect.com/science/article/B6VP8-4F8SXDF-8/2/ab8528c05881b60240a52439bf30ff89

Objective: Ceramide, an intermediate of apoptosis induction in response to chemotherapy, can be detoxified by glycosylation at the cytoplasmic surface of the Golgi membrane. P-glycoprotein (p-gp) might augment ceramide glycosylation by translocating glucosylceramide (GC) across the Golgi membrane. We aimed to show that glucosylceramide synthase (GCS) activity is linked to p-gp expression and resistance to ceramide-induced apoptosis in acute myeloid leukemia (AML).

Methods: Apoptosis and cell-cycle analysis were measured using propidium iodide staining and flow cytometry. Fluorescent microscopy assessed p-gp expression in, and rhodamine 123 uptake by, the Golgi. P-gp interaction with GC was assessed by modulation of rhodamine accumulation. The GCS activity assay was based upon the transfer of UDP-3H-glucose to C8-ceramide to form radiolabeled GC, by rate-limiting cell-derived GCS. TLC and fluorimetry were used to measure the metabolites of fluorescent ceramide. Cell viability was measured using 7-amino-actinomycin D staining and flow cytometry with an internal standard for cell enumeration.

Results: P-gp+ cell lines (KG1a, TF-1) were resistant to C8-ceramide-induced apoptosis compared to p-gp- cell lines (HL-60, U937). P-gp inhibitors GF120918 and cyclosporin A enhanced ceramide-induced apoptosis in the p-gp expressing cells. P-gp expression was identified in the Golgi of these cells. Pgp's efflux function in TF-1 but not KG1a cells was inhibited by glucosylceramide. In the presence of p-gp inhibitors, R123 accumulation in the Golgi of TF-1 cells was lost, and GCS activity and lactosylceramide formation were downregulated. Intact cells were necessary for the involvement of p-gp in the regulation of GCS activity.

Conclusion: Our data suggests that ceramide induces apoptosis in AML cells and that p-gp confers resistance to ceramide-induced apoptosis, with modulation of the ceramide-glucosylceramide pathway making a marked contribution to this resistance in TF-1 cells.


http://www.sciencedirect.com/science/article/B6VP8-428FKDM-F/2/4d72eb835879c26bce01c9f7ff3984e7

Objective: The aim of this study was to determine the molecular basis of p47-phox-deficient chronic granulomatous disease (CGD), the most common autosomal recessive form of the disease. CGD is an inherited condition characterized by defective oxygen radical production due to defects in the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Mutational analysis of p47-phox-deficient CGD patients previously demonstrated that the majority of patients have a GT dinucleotide ([Delta]GT) deletion at the start of exon 2, a signature sequence also observed in the highly homologous pseudogenes of NCF1.

Materials and Methods: We performed genetic analysis of NCF1 and its pseudogenes using genomic DNA in 29 p47-phox-deficient CGD patients from 22 separate families. First-strand cDNA analysis was performed in 17 of the 29 patients.

Results: We confirmed the significance of the [Delta]GT mutation; in 27 of 29 patients, only the [Delta]GT sequence was detectable. All but one of the 27 had at least one additional signature sequence, specific to the pseudogene, in either intron 1 and/or intron 2. We extended our analysis to look at signature sequence differences in exons 6
and 9 and detected both the wild-type and pseudogene sequences in all patients tested.

Conclusions Although detection of only [Delta]GT sequence accounts for over 85% of affected patients, the molecular basis is most likely due to partial cross-over events between the wild-type and pseudogene(s) of p47-phox at different recombination sites. Our results suggest that complete gene conversion or deletion of the p47-phox gene (NCF1) occurs rarely, if at all.


Several lines of evidence suggest that overexpression of interferon [gamma] (IFN-[gamma]) in the marrow microenvironment may play a role in the pathogenesis of marrow suppression in aplastic anemia. We previously showed that overexpression of IFN-[gamma] by marrow stromal cells inhibits human long-term culture initiating cell activity assayed in vitro to a much greater degree than the addition of soluble IFN-[gamma]. The effect of IFN-[gamma] on true repopulating stem cells assayed in vivo has not been studied previously. We compared the effect of co-culture of murine marrow cells in the presence of stromal cells transduced with a retroviral vector...
expressing murine IFN-[gamma] vs stromal cells transduced with a control neo vector. Using a murine congenic competitive repopulation assay, there was significantly less long-term repopulating stem cell activity remaining after culture on mIFN-[gamma]-expressing stroma as compared to control stroma. We also investigated the effect of directly transducing murine bone marrow cells with the mIFN-[gamma] or control vector. Marrow cells transduced with either vector were transplanted into W/Wv recipient mice. The percentage of vector-containing cells in the mIFN-[gamma] mice was significantly lower than in the control mice, suggesting that mIFN-[gamma]-transduced primitive cells may not have survived culture, or that mIFN-[gamma] directly decreases gene transfer into repopulating cells. Despite no significant differences in white or red blood cells in the mice transplanted with the mIFN-[gamma]-transduced cells, the number of bone marrow colony-forming unit-C 16 weeks after transplantation was significantly lower in the IFN-[gamma] group. These data indicate that ectopic or overexpression of mIFN-[gamma], especially by marrow microenvironmental elements, may have a marked effect on primitive hematopoiesis as assayed in vivo. Published by Elsevier Science Inc.

Experimental Neurology (6)


Bone marrow (BM) is a rich source of stem cells and may represent a valid alternative to neural or embryonic cells in replacing autologous damaged tissues for neurodegenerative diseases. The purpose of the present study is to identify human adult BM progenitor cells capable of neuro-glial differentiation and to develop effective protocols of trans-differentiation to surmount the hematopoietic commitment in vitro. Heterogeneous cell populations such as whole BM, low-density mononuclear and mesenchymal stem (MSCs), and several immunomagnetically separated cell populations were investigated. Among them, MSCs and CD90+ cells were demonstrated to express neuro-glial transcripts before any treatment. Several culture conditions with the addition of stem cell or astroblast conditioned media, different concentrations of serum, growth factors, and supplements, used alone or in combinations, were demonstrated to alter the cellular morphology in some cell subpopulations. In particular, MSCs and CD90+ cells acquired astrocytic and neuron-like morphologies in specific culture conditions. They expressed several neuro-glial specific markers by RT-PCR and glial fibrillary acid protein by immunocytochemistry after co-culture with astroblasts, both in the absence or presence of cell contact. In addition, floating neurosphere-like clones have been observed when CD90+ cells were grown in neural specific media. In conclusion, among the large variety of human adult BM cell populations analyzed, we demonstrated the in vitro neuro-glial potential of both the MSC and CD90+ subset of cells. Moreover, unidentified soluble factors provided by the conditioned media and cellular contacts in co-culture systems were effective in inducing the neuro-glial phenotype, further supporting the adult BM neural differentiative capability.

Abnormal formation or loss of myelin is a distinguishing feature of many neurological disorders and contributes to the pathobiology of neurotrauma. In this study we characterize the functional and molecular changes in CNS white matter in Long Evans Shaker (LES) rats. These rats have a spontaneous mutation of the gene encoding myelin basic protein which results in severe dysmyelination of the central nervous system (CNS), providing a unique model for demyelinating/dysmyelinating disorders. To date, the functional and molecular changes in CNS white matter in this model are not well understood. We have used in vivo somatosensory evoked potential (SSEP), in vitro compound action potential (CAP) recording in isolated dorsal columns, confocal immunohistochemistry, Western blotting and real-time PCR to examine the electrophysiological, molecular and cellular changes in spinal cord white matter in LES rats. We observed that dysmyelination is associated with dispersed labeling of Kv1.1 and Kv1.2 K⁺ channel subunits, as well as Caspr, a protein normally confined to paranodes, along the LES rat spinal cord axons. Abnormal electrophysiological properties including attenuation of CAP amplitude and conduction velocity, high frequency conduction failure and enhanced sensitivity to K⁺ channel blockers 4-aminopyridine and dendrotoxin-I were observed in spinal cord axons from LES rats. Our results in LES rats clarify some of the key molecular, cellular and functional consequences of dysmyelination and myelin-axon interactions. Further understanding of these issues in this model could provide critical insights for neurological disorders characterized by demyelination.


Green tea polyphenol is known to act as a buffer, reducing biological responses to oxidative stress. Several effects of polyphenol have been reported, such as protection of tissue from ischemia, antineoplastic and anti-inflammatory effects, and suppression of arteriosclerosis. In this study, we investigated whether peripheral nerve segments could be kept viable in a polyphenol solution for 1 month. Sciatic nerve segments, 20 mm long, were harvested from Lewis rats and treated in three different ways before transplanting to recipient Lewis rats to bridge sciatic nerve gaps created by removal of 15-mm-long nerve segments. Group F: nerve segments were transplanted immediately after harvesting. Group P: nerve segments were transplanted after they had been stored in Dulbecco's Modified Eagle's Medium (DMEM) containing polyphenol for 7 days at 4[°]C and then in DMEM for 21 days at 4[°]C. Group M: nerve segments were stored in DMEM solution alone for 28 days at 4[°]C. Viability of the nerve segments was assessed by vital staining (calcein-AM/ethidium homodimer), by electron microscopy and by genomic studies before transplantation. Nerve regeneration was evaluated using electrophysiological and morphological studies 12 and 24 weeks after transplantation. Neural cell viability of the preserved nerve segments was confirmed in group P, in which the nerve regeneration was similar to that in group F and superior to that in group M. Peripheral nerve segments can be successfully preserved for 1 month using green tea polyphenol.


http://www.sciencedirect.com/science/article/B6WFG-49CKWMS-1/2/e1819cd1b98f66deee409dd4781d84d3

The neuropilins, NP-1 and NP-2, are coreceptors for Sema3A and Sema3F, respectively, both of
which are repulsive axonal guidance molecules. NP-1 and NP-2 are also coreceptors for vascular endothelial growth factor (VEGF). The neuropilins and their ligands are known to play prominent roles in axonal pathfinding, fasciculation, and blood vessel formation during peripheral nervous system (PNS) development. We confirmed a prior report (Exp. Neurol. 172 (2001) 398) that VEGF mRNA levels rise during Wallerian degeneration in the PNS and herein demonstrate that NP-1, NP-2, Sema3A, and Sema3F mRNA levels increase in peripheral nerves distal to a transection or crush injury. In a sciatic nerve crush model, in which axonal regeneration is robust, the highest levels of Sema3F mRNA below the injury site are in the epi- and perineurium. Our results suggest the possibility that the neuropilins and their semaphorin ligands serve to guide, rather than to impede, regenerating axons in the adult PNS.


http://www.sciencedirect.com/science/article/B6WFG-4C8H7KS-9J/2/ddde22a10d94211d246e58ba61c16d08

The adrenal gland is a well-demonstrated source for different neurotrophic factors. The presence of the [beta]-nerve growth factor ([beta]-NGF) mRNA in the adrenal tissue used for grafting in a Parkinsonian patient is reported here. Adrenal samples were obtained on the day of implantation, and a specific cDNA was synthesized after the extraction of total RNA using a synthetic oligonucleotide as a reverse transcription primer. A 168-bp portion of the cDNA was amplified using two other oligonucleotides as Taq polymerase primers in a polymerase chain reaction. Thirty-two cycles of amplification were performed. The amplification products were identified by agarose gel electrophoresis and Southern blot analysis as a single DNA band hybridizing with a third [beta]-NGF specific oligonucleotide. The identity of the fragment was confirmed by DNA sequencing. Quantitative analysis demonstrated a [beta]-NGF mRNA concentration exceeding 5 fg/[mu]g of total adrenal RNA. These findings add NGF to the other neurotrophic factors produced by the gland (i.e., basic fibroblast growth factor) and demonstrate the retained functional capacity of the Parkinsonian adrenal to express the [beta]-NGF mRNA. All these data may assume relevant meaning for neurotransplantation research.


http://www.sciencedirect.com/science/article/B6WFG-49Y3XT1-8/2/ef8ceedf4eee3ea8aedb56d13c386860

Bone marrow stromal cells (BMSC) have been shown to generate neural cells under experimental conditions in vitro and following transplantation into animal models of stroke and traumatic CNS injury. Hastened recovery from the neurological deficit has not correlated with structural repair of the lesion in the stroke model. Secretory functions of BMSC, such as the elaboration of growth factors and cytokines, have been hypothesized to play a role in the enhanced recovery of neurological function. Using gene expression arrays, real time RT-PCR and radioimmunoassay, we have found that brain natriuretic peptide (BNP) is synthesized and released by BMSC at physiologically relevant levels in vitro. BNP, like its close homolog atrial natriuretic peptide (ANP), exerts powerful natriuretic, diuretic and vasodilatory effects. We speculate that transplanted BMSCs facilitate recovery from brain and spinal cord lesions by releasing BNP and other vasoactive factors that reduce edema, decrease intracranial pressure and improve cerebral perfusion.

http://www.sciencedirect.com/science/article/B6WFH-45TDHV0-7/2/ce01ef4158c85f4f014650cf6e888693


http://www.sciencedirect.com/science/article/B6WFH-4F94YMS-2/2/f9a217c4e08e43ab497c0c29532b703c

The sporocyst stage of trematode development plays the crucial role of establishing a successful infection in the molluscan intermediate host. Due to the small size and presence of this stage within the tissues of the host, much of our current knowledge of sporocyst biology relies on cultured specimens. To gain insight into the transcriptional patterns of early sporocysts, suppression subtractive hybridization was employed to identify 69 unique expressed sequence tags likely to be upregulated in cultured sporocysts of Echinostoma paraensei, a trematode parasite of the planorbid snail, Biomphalaria glabrata. Upwards of 70% of the unique sequences were not identified by homology to known genes. However, one transcript may encode an inhibitor of nitric oxide synthase, indicating a possible role in protection against host defense mechanisms. An array containing the majority of the sequenced clones was probed with in vivo-derived cDNA, confirming for the first time in vivo expression of putative sporocyst genes. However, qPCR quantification demonstrated significant reductions in transcription rates in cultured versus in vivo sporocysts for three of six transcripts tested. Additionally, five of the six tested transcripts demonstrated significant variation in expression over the entire life cycle, with the significant upregulation occurring during early intramolluscan development or in the free-living stages immediately preceding snail penetration, confirming the efficacy of the SSH technique.


http://www.sciencedirect.com/science/article/B6WFH-47T8BJ8-2/2/d51ab84f57e92a2e3b552258e839be2c

The rapid emergence of multidrug-resistant Plasmodium falciparum is a worldwide concern. Despite the magnitude of the problem, the mechanisms involved in this phenomenon are not well understood. One current proposal suggests that toxic heme molecules are degraded by glutathione (GSH), and that anti-malarial drugs, such as chloroquine (CQ), inhibit this degradation, thus implicating GSH in drug resistance. Furthermore, in some strains of Plasmodium berghei and P. falciparum, chloroquine resistance is accompanied by an increase in glutathione levels and increased activity in GSH-related enzymes. We are investigating the relationship between the [gamma]-glutamylcysteine synthetase (ggcs) gene, the rate-limiting
enzyme in de novo synthesis of GSH, and drug resistance in P. berghei at the molecular level. In this report, we have demonstrated an increase in pbggcs mRNA levels associated with CQ and mefloquine (MFQ) resistance. In addition, the pbggcs gene locus structure was shown to be similar and localized to chromosome 8 in four parasite lines of P. berghei with different drug resistance profiles. This work suggests a link between increased GSH levels and drug resistance in Plasmodium.

Index Descriptors and Abbreviations: Apicomplexa; Malaria; Plasmodium berghei; Drug resistance; [gamma]-Glutamylcysteine synthetase; Glutathione; Chloroquine; MDR, multidrug resistance phenotype; AMO, amodiaquine; MFQ, mefloquine; CQ, chloroquine; GSH, glutathione; ggcs, [gamma]-glutamylcysteine synthetase; GB, GenBank; RBC, red blood cell; BSO, buthionine sulfoximine; pfggcs, Plasmodium falciparum ggcs gene; pbggcs, Plasmodium berghei ggcs gene; RPA, ribonuclease protection assay.


http://www.sciencedirect.com/science/article/B6WFH-48W2R7B-1/2/74ba10e22ca9bd473693dd797c82dedf

In this study, the mature domains of type I (CPB) and type II (CPA) cysteine proteinases (CPs) of Leishmania infantum were expressed and their immunogenic properties defined using sera from active and recovered cases of human visceral leishmaniasis and sera from infected dogs. Immunoblotting and ELISA analysis indicated that a freeze/thaw extract of parasite antigens showed similar and intensive recognition in both active cases of human and dog sera but lower recognition in recovered human individuals. The total IgG of actively infected human sera was higher than in recovered cases when rCPs were used as antigen. In contrast to dog sera, both active and recovered human cases have higher recognition toward rCPB than rCPA. Furthermore, the asymptomatic dogs in contrast to the symptomatic cases exhibited specific lymphocyte proliferation to both crude antigens and rCPs.


http://www.sciencedirect.com/science/article/B6WFH-4F7YMVY-1/2/81582cf47910cd53467d4bcd56e158be

The objective of this study was to analyse the modulatory effect of proteins released by cultured Leishmania infantum promastigotes on the cellular immune response of infected susceptible (BALB/c) and more resistant (C57BL/6) mice strains after 30 and 45 days of infection. One month after parasite inoculation, L. infantum released protein fractions (High, Inter, and Low according to molecular weight) stimulated C57BL/6 mice spleen cells to proliferate and to express cytokines. Following the decrease of parasite load only the Low protein fraction induced a considerable release of IL-4. In BALB/c mice, specific immune response to protein fractions was only observed at the higher parasitic level, with the fraction Inter promoting the production of IL-4 and fractions High and Low inducing high levels of IL-12. These results point out to a role of these proteins fractions in the modulation of host immunity, that depending on the host genetic background and parasite magnitude, seem to be critical in the control of parasite replication levels, thus avoiding premature host death.

http://www.fasebj.org/cgi/content/abstract/01-0602fjev1

A pharmacological approach to neoplasia by differentiation therapy relies on the availability of cytodifferentiating agents whose antitumor efficacy is usually assayed first on malignant cells in vitro. Using murine erythroleukemia cells (MELCs) as the model, we found that WEB-2086, a triazolobenzodiazepine-derived PAF antagonist originally developed as an anti-inflammatory drug, induces a dose-dependent inhibition of MELC growth and hemoglobin accumulation as a result of a true commitment to differentiation. MELCs treated for 5 days with 1 mM WEB-2086 show greater than or equal to 85% benzidine-positive cells, increased expression of a- and b-globin genes, and down-regulation of c-Myb. This differentiation pattern, which does not involve histone H4 acetylation and is abrogated by the action of phorbol 12-myristate 13-acetate, recalls the pattern induced by hexamethylene bisacetamide (HMBA). In addition to MELCs, human erythroleukemia K562 and HEL and myeloid HL60 cells are massively committed to maturation by WEB-2086 and, with some differences, by its analog, WEB-2170. This suggests that WEB-2086, structurally distant from other known inducers, might be a member of a new class of cytodifferentiation agents active on a broad range of transformed cells in vitro and useful, prospectively, for anticancer therapy due to their high tolerability in vivo. Key words: inducer · PAF receptor · maturation · neoplasia · differentiation therapy


http://www.fasebj.org/cgi/content/abstract/02-0883fjev1

Hydrogen sulfide (H2S), produced by commensal sulfate-reducing bacteria, is an environmental insult that potentially contributes to chronic intestinal epithelial disorders. We tested the hypothesis that exposure of nontransformed intestinal epithelial cells (IEC-18) to the reducing agent sodium hydrogen sulfide (NaHS) activates molecular pathways that underlie epithelial hyperplasia, a phenotype common to both ulcerative colitis (UC) and colorectal cancer. Exposure of IEC-18 cells to NaHS rapidly increased the NADPH/NADP ratio, reduced the intracellular redox environment, and inhibited mitochondrial respiratory activity. The addition of 0.2-5 mM NaHS for 4 h increased the IEC-18 proliferative cell fraction (P<0.05), as evidenced by analysis of the cell cycle and proliferating cell nuclear antigen expression, while apoptosis occurred only at the highest concentration of NaHS. Thirty minutes of NaHS exposure increased (P<0.05) c-Jun mRNA concentrations, consistent with the observed activation of mitogen activated protein kinases (MAPK). Microarray analysis confirmed an increase (P<0.05) in MAPK-mediated proliferative activity, likely reflecting the reduced redox environment of NaHS-treated cells. These data identify functional pathways by which H2S may initiate epithelial dysregulation and thereby contribute to UC or colorectal cancer. Thus, it becomes crucial to understand how genetic background may affect epithelial responsiveness to this bacterial-derived environmental insult. Key words: IEC-18 colorectal cancer ulcerative colitis epithelial hyperproliferation microarray analysis
A role for choline during early stages of mammalian embryogenesis has not been established, although recent studies show that inhibitors of choline uptake and metabolism, 2-dimethylaminoethanol (DMAE), and 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (ET-18-OCH3), produce neural tube defects in mouse embryos grown in vitro. To determine potential mechanisms responsible for these abnormalities, choline metabolism in the presence or absence of these inhibitors was evaluated in cultured, neurulating mouse embryos by using chromatographic techniques. Results showed that 90%-95% of 14C-choline was incorporated into phosphocholine and phosphatidylcholine (PtdCho), which was metabolized to sphingomyelin. Choline was oxidized to betaine, and betaine homocysteine methyltransferase was expressed. Acetylcholine was synthesized in yolk sacs, but 70 kDa choline acetyltransferase was undetectable by immunoblot. DMAE reduced embryonic choline uptake and inhibited phosphocholine, PtdCho, phosphatidylethanolamine (PtdEtn), and sphingomyelin synthesis. ET-18-OCH3 also inhibited PtdCho synthesis. In embryos and yolk sacs incubated with 3H-ethanolamine, 95% of recovered label was PtdEtn, but PtdEtn was not converted to PtdCho, which suggested that phosphatidylethanolamine methyltransferase (PeMT) activity was absent. In ET-18-OCH3-treated yolk sacs, PtdEtn was increased, but PtdCho was still not generated through PeMT. Results suggest that endogenous PtdCho synthesis is important during neurulation and that perturbed choline metabolism contributes to neural tube defects produced by DMAE and ET-18-OCH3.

Key Words: neurulation · dimethylaminoethanol · ET-18-OCH3 · embryo culture

Substance P (SP), a potent modulator of neuroimmunoregulation, is expressed in human immune cells. We observed elevated plasma SP levels in HIV-infected men compared with uninfected subjects. In the present study, we investigated the possible cellular source of the increased SP level caused by HIV infection. Using real-time reverse transcriptase-polymerase chain reaction, we demonstrated that monocyte-derived macrophages (MDM) and lymphocytes from both placental cord blood and adult peripheral blood expressed SP mRNA, which was significantly increased by HIV infection. HIV-induced SP expression was positively related to virus replication in the infected MDM. Purified recombinant HIV envelope glycoprotein 120 (gp120) derived from both the macrophage-tropic strain (MN) and the T lymphocyte-tropic strain (IIIB), when added to MDM cultures, enhanced SP mRNA expression. The gp120-induced SP expression was abrogated by pretreating the cells with soluble CD4. Furthermore, the activation of HIV in the latently infected promonocytic cell line (U1) and T-cell line (ACH-2) up-regulated SP mRNA expression. These data support the hypothesis that interaction of HIV and SP may have significant in vivo relevance to the immunopathogenesis of HIV infection and AIDS. Key words: lymphocytes · gp120 · tachykinin · neuroimmunoregulation · monocyte-derived macrophages
Junctin is a 26-kDa integral membrane protein, colocalized with the ryanodine receptor (RyR) and calsequestrin at the junctional sarcoplasmic reticulum (SR) membrane in cardiac and skeletal muscles. To elucidate the functional role of junctin in heart, transgenic (TG) mice overexpressing canine junctin (24-29 folds) under the control of mouse a-myosin heavy chain promoter were generated. Overexpression of the junctin in mouse heart was associated with heart enlargements, bradycardia, atrial fibrillation, and increased fibrosis. Many ultrastructural alterations were observed in TG atria. The junctional SR cisternae facing transverse-tubules contained a dense matrix of calsequestrin in TG heart. According to echocardiography, TG mice showed enlarged left ventricles, dilated right atriums, and ventricles with paradoxical septal motion and impaired left ventricular systolic function. Overexpression of junctin led to down-regulation of triadin and RyR but to up-regulation of dihydropyridine receptor. The L-type Ca2+ current density and action potential durations increased, which could be the cause for the bradycardia in TG heart. This study provides an important example of pathogenesis leading to substantial cardiac remodeling and atrial fibrillation, which was caused by overexpression of junctin in heart. Key words: excitation-contraction coupling · sarcoplasmic reticulum · ryanodine receptor · dihydropyridine receptor


http://www.fasebj.org/cgi/content/abstract/01-0354fjev1

The effect of uni-axial cyclic mechanical stretch on the activation of the transcription factor nuclear factor kB (NF-kB) was investigated in a human fibroblast cell line (TIG-1). In response to uni-axial cyclic stretch, NF-kB was found to be translocated into the nucleus. The NF-kB was first detectable 2 min after the onset of stretch and then peaked at 4 min and returned to the basal level within 10 min. To investigate whether NF-kB is activated following the translocation into the nucleus, we measured the luciferase activity in the cells transfected with pNF-kB-luciferase. The activity of luciferase increased 4 min after the initiation of cyclic stretch, peaked at 15 min (6.4-fold increase), and decreased gradually. We examined the involvement of the stretch-activated (SA) channel in the stretch-induced NF-kB activation. The application of Gd3+, a blocker of the SA channel, or the removal of extracellular Ca2+ inhibited both the translocation into the nucleus and the activation of NF-kB, which suggests that NF-kB is activated by uni-axial cyclic stretch via SA channel activation in human lung fibroblasts. Key words: stretch-activated channel · gadolinium · calcium · fibroblast


http://www.fasebj.org/cgi/content/abstract/03-0773fjev1

Nuclear factor (NF)-B p50 protein is involved in promoting survival in hippocampal neurons after trimethyltin (TMT)-injury. In the current study, hippocampal NF-B activity was examined and quantitated from transgenic B-lacZ reporter mice after chemical-induced injury. NF-B activity was localized primarily to hippocampal neurons and significantly elevated over that in saline-treated mice between 4 and 21 days after TMT injection. Seven days after TMT injection, a timepoint of elevated NF-B activity, gene expression in the hippocampus was studied by microarray analysis through comparison of expression profiles between treated nontransgenic and p50-null mice with their saline-injected controls. Seventeen genes increased in nontransgenic TMT-treated mice relative to saline-treated as well as showing no increase in p50-null mice, indicating a role for p50 in their regulation. One of these genes, the Na+, K+-ATPase- subunit, was detected in brain for the first time. Several of the genes modulated by NF-B are potentially related to neuroplasticity,
providing additional evidence that this transcription factor is a neuroprotective signal in the hippocampus. Key words: signal transduction Na+, K+-ATPase neurodegeneration NF-B p50 transcription factors


http://www.fasebj.org/cgi/content/abstract/01-0940fjev1

Proprotein convertases (PCs) are evolutionarily conserved enzymes responsible for processing the precursors of many bioactive peptides in mammals. The invertebrate homologues of PC2 play important roles during development that makes the enzyme a good target for practical applications in pest management. Screening of a plant nematode Heterodera glycines cDNA library resulted in isolation of a full-length clone encoding a PC2-like precursor. The deduced protein (74.2 kD) exhibits strong amino acid homology to all known PC2s, including human, and shares the main structural characteristics: signal peptide; prosegment; catalytic domain, with D/H/S catalytic triad, PC2-specific residues, and 7B2 binding sites; P domain (with RRGDT pentapeptide); and carboxyl terminus. Comparative analysis of PC2s from 15 species discloses the presence of an insert in the catalytic domain unique to nematodes. Expression of PC2-like mRNA found in eggs and juveniles was undetectable in adult stages of H. glycines. Nucleotide analysis reveals distinctive differences in base composition and codon usage between H. glycines and Caenorhabditis elegans PC2s. The H. glycines cDNA clone encoding PC2 is the first one isolated from plant-parasitic nematodes. Key words: cDNA cloning · (G+C) content · plant-parasitic nematode · Heterodera glycines


http://www.fasebj.org/cgi/content/abstract/02-0243fjev1

Glucose-dependent insulinotropic polypeptide (GIP) is secreted postprandially and acts in concert with glucose to stimulate insulin secretion from the pancreas. Here, we describe a novel pathway for the regulation of GIP receptor (GIPR) expression within clonal b-cell lines, pancreatic islets, and in vivo. High (25 mM) glucose was able to significantly reduce GIPR mRNA levels in INS(832/13) cells after only 6 h. In contrast, palmitic acid (2 mM) and WY 14643 (100 mM) stimulated approximate doublings of GIPR expression in INS(832/13) cells under low (5.5 mM), but not high (25 mM), glucose conditions, suggesting that fat can regulate GIPR expression via PPARa in a glucose-dependent manner. Both MK-886, an antagonist of PPARa, and a dominant negative form of PPARa transfected into INS(832/13) cells caused a significant reduction in GIPR expression in low, but not high, glucose conditions. Finally, in hyperglycemic clamped rats, there was a 70% reduction in GIPR expression in the islets and a 71% reduction in GIP-stimulated insulin secretion from the perfused pancreas. Thus, evidence is presented that the GIPR is controlled at normoglycemia by the fatty acid load on the islet; however, when exposed to hyperglycemic conditions, the GIPR is down-regulated, which may contribute to the decreased responsiveness to GIP that is observed in type 2 diabetes. Key words: PPARa · type 2 diabetes · Zucker rats · INS(832/13) cells · gastric inhibitory polypeptide

Reactive oxygen species such as superoxide and hydroxyl radicals have been implicated in the pathogenic growth of various cell types. The molecular mechanisms involved in redox-sensitive cell growth control are poorly understood. Stimulation of cultured vascular smooth muscle cells (VSMC) with xanthin/xanthin oxidase (X/XO) increases proliferation, whereas stimulation with hydrogen peroxide and Fe3+NTA (H-Fe) causes growth arrest of VSMC. Differential Display led to the identification of two novel, differentially regulated redox-sensitive genes. The dominant negative helix-loop-helix protein Id3 is induced by X/XO and down-regulated by H-Fe. The transcription factor gut-enriched Kruppel-like factor (GKLF) is induced by H-Fe but not by X/XO. Induction of GKLF and inhibition of Id3 via transfection experiments leads to growth arrest, whereas overexpression of Id3 and inhibition of GKLF cause cell growth. Id3 down-regulation is induced via binding of GKLF to the Id3 promoter and concomitantly reduced Id3 gene transcription rate. GKLF induction by H-Fe is mediated through hydroxyl radicals, p38MAP kinase-, calcium-, and protein synthesis-dependent pathways. Id3 is induced by X/XO via superoxide, calcium, p38, and p42/44 MAP kinase. GKLF induces and Id3 depresses expression of p21WAF1/Cip1, p27KIP1, p53. Induction of Id3 is accomplished by angiotensin II via superoxide release. A vascular injury mouse model revealed that Id3 is overexpressed in proliferating vascular tissue in vivo. These findings reveal novel mechanisms of redox-controlled cellular proliferation involving GKLF and Id3 that may have general implications for our understanding of vascular and nonvascular growth control.--Nickenig, G., Baudler, S., Muller, C., Werner, N., Welzel, H., Strehlow, K., Bohm, M. redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 in vitro and in vivo.


Interleukin 1{beta} (IL-1{beta}) is a proinflammatory cytokine that maintains thermal hyperalgesia and facilitates the release of calcitonin gene-related peptide from rat cutaneous nociceptors in vivo and in vitro. Brief applications of IL-1{beta} to nociceptive neurons yielded a potentiation of heat-activated inward currents (I heat) and a shift of activation threshold toward lower temperature without altering intracellular calcium levels. The IL-1{beta}-induced heat sensitization was not dependent on G-protein-coupled receptors but was mediated by activation of protein kinases. The nonspecific protein kinase inhibitor staurosporine, the specific protein kinase C inhibitor bisindolylmaleimide BIM1, and the protein tyrosine kinase inhibitor genistein reduced the sensitizing effect of IL-1{beta} whereas negative controls were ineffective. RT-PCR and in situ hybridization revealed IL-1RI but not RII expression in neurons rather than surrounding satellite cells in rat dorsal root ganglia. IL-1{beta} acts on sensory neurons to increase their susceptibility for noxious heat via an IL-1RI/PTK/PKC-dependent mechanism.--Obreja, O., Rathee, P. K., Lips, K. S., Distler, C., Kress, M. IL-1{beta} potentiates heat-activated currents in rat sensory neurons: involvement of IL-1RI, tyrosine kinase, and protein kinase C.


Consumption of red meat is associated with increased colon cancer risk. Our previous work
indicated that this association might be due to the heme content of red meat. In rat studies, dietary heme increased colonic cytotoxicity and epithelial cell turnover, carcinogenesis biomarkers. Here we apply DNA microarray technology to examine effects of heme on colonic gene expression. A rat colon-specific microarray was constructed and hybridized in duplicate to RNA extracts from colon scrapings of rats fed diets with or without heme (n=6-7). We were able to reproducibly identify changes in colonic mRNA abundance in response to heme. Most striking was a >10-fold down-regulation of a single rat gene, an unprecedented gene-modulating effect of a dietary component. Based on homology, the novel gene encodes a pentraxin, the first identified in colon. Pentraxins are postulated to be involved in dealing with dying cells. Quantitative PCR confirmed the strong heme-induced down-regulation of this gene, which we named mucosal pentraxin (Mptx). Overall, our data support the efficacy of cDNA array expression profiling to investigate effects of specific nutrients in an in vivo system and may provide an approach to establishing markers for diet-induced stress of mammalian colonic mucosa.--van der Meer-van Kraaij, C., van Lieshout, E. M. M., Kramer, E., van der Meer, R., Keijer, J. Mucosal pentraxin (Mptx), a novel rat gene 10-fold down-regulated in colon by dietary heme.

**FEBS J.** (2)


http://content.febsjournal.org/cgi/content/abstract/272/4/942

Planarians are one of the simplest animal groups with a central nervous system. Their primitive central nervous system produces large quantities of a variety of neuropeptides, of which many are amidated at their C terminus. In vertebrates, peptide amidation is catalyzed by two enzymes [peptidylglycine {alpha}-hydroxylating monooxygenase (PHM) and peptidyl-{alpha}-hydroxylglycine {alpha}-amidating lyase] acting sequentially. In mammals, both enzymatic activities are contained within a single protein that is encoded by a single gene. By utilizing PCR with degenerate oligonucleotides derived from conserved regions of PHM, we succeeded in cloning a full-length cDNA encoding planarian PHM. The deduced amino acid sequence showed full conservation of five His residues and one Met residue, which bind two Cu atoms that are essential for the activity of PHM. Northern blot analysis confirmed the expression of a PHM mRNA of the expected size. Distribution of the mRNA was analyzed by in situ hybridization, showing specific expression in neurons with two morphologically distinct structures, a pair of the ventral nerve cords and the brain. The distribution of PHM was very similar to that of cytochrome b561. This indicates that the ascorbate-related electron transfer system operates in the planarian central nervous system to support the PHM activity and that it predates the emergence of Plathelminthes in the evolutionary history.


http://content.febsjournal.org/cgi/content/abstract/272/6/1440
The upp gene, encoding uracil phosphoribosyltransferase (UPRTase) from the thermoacidophilic archaeon Sulfolobus solfataricus, was cloned and expressed in Escherichia coli. The enzyme was purified to homogeneity. It behaved as a tetramer in solution and showed optimal activity at pH 5.5 when assayed at 60 {degrees}C. Enzyme activity was strongly stimulated by GTP and inhibited by CTP. GTP caused an approximately 20-fold increase in the turnover number kcat and raised the Km values for 5-phosphoribosyl-1-diphosphate (PRPP) and uracil by two- and >10-fold, respectively. The inhibition by CTP was complex as it depended on the presence of the reaction product UMP. Neither CTP nor UMP were strong inhibitors of the enzyme, but when present in combination their inhibition was extremely powerful. Ligand binding analyses showed that GTP and PRPP bind cooperatively to the enzyme and that the inhibitors CTP and UMP can be bound simultaneously (KD equal to 2 and 0.5 (micro)M, respectively). The binding of each of the inhibitors was incompatible with binding of PRPP or GTP. The data indicate that UPRTase undergoes a transition from a weakly active or inactive T-state, favored by binding of UMP and CTP, to an active R-state, favored by binding of GTP and PRPP.

FEMS Immunology and Medical Microbiology(28)


The inhibitory effect of human and porcine bile samples to detect Helicobacter DNA was studied by adding different concentrations of bile samples to PCR mixtures of six thermostable DNA polymerases containing cagA specific primers and Helicobacter pylori DNA. PCR products were amplified by using the Rotorgene system and SYBR Green I. Among the six DNA polymerases tested, rTth had the lowest sensitivity to bile inhibitors, whereas Taq and Tfi had the highest sensitivity. Bile proteins did not inhibit AmpliTaq DNA polymerase, whereas the fraction containing mainly bile acids and their salts inhibited the amplification capacity of AmpliTaq. Heating human bile at 98 [deg]C and adding casein and formamide to the reaction mixture reduced the PCR inhibitory effect of bile. Therefore, a pre-PCR treatment based on dilution and heating of bile, adding casein and formamide to the reaction mixture of rTth DNA polymerase was found efficient to amplify DNA directly in bile.


http://www.sciencedirect.com/science/article/B6T2T-3YB4D8T-9/2/96b489e3e9c3532825cb28b5c4bd3aab

The promising arena of DNA-based vaccines has led us to investigate possible candidates for immunization against bacterial pathogens. One such target is the opportunistic pathogen Pseudomonas aeruginosa which produces exotoxin A (PE), a well-characterized virulence factor encoded by the toxA gene. In its native protein form, PE is highly cytotoxic for susceptible eukaryotic cells through ADP-ribosylation of elongation factor-2 following internalization and processing of the toxin. To study the biologic and immunological effects of PE following in situ expression, we have constructed eukaryotic plasmid expression vectors containing either the
wild-type or a mutated, non-cytotoxic toxA gene. In vitro analysis by transfection of UM449 cells suggests that expression of the wild-type toxA gene is lethal for transfected cells whereas transfection with a mutated toxA gene results in the production of inactive PE which can be readily detected by immunoblot analysis of cell lysates. To investigate the effects resulting from the intracellular expression of potentially cytotoxic gene products in DNA vaccine constructs, we immunized mice with both the wild-type and mutant toxA plasmid constructs and analyzed the resulting humoral and cellular immune responses. Immunization with the mutated toxA gene results in production of neutralizing antibodies against native PE and potentiates a TH1-type response, whereas only a minimal humoral response can be detected in mice immunized with wild-type toxA. DNA-based vaccination with the non-cytotoxic toxAmut gene confers complete protection against challenge with the wild-type PE. Therefore, genetic immunization with genes encoding potentially cytotoxic gene products raises concern with regard to the selection of feasible gene targets for DNA vaccine development.


http://www.sciencedirect.com/science/article/B6T2T-45XRJ79-2/2/200df3f11a690e5abaec319f9daf68eb

In order to estimate the rate of microsporidia, cryptosporidia and giardia contamination of swimming pools, sequential samples of water were collected during a one-year period in six different swimming pools in Paris, France. Forty-eight samples were submitted to filtrations. Eluates were examined for microsporidia using polymerase chain reaction (PCR) and for cryptosporidia and giardia using immunofluorescence staining. One of 48 specimens was positive for microsporidia. Using DNA sequence analysis, unknown microsporidia species were identified, which were close to an insect microsporidia Endoreticulatus schubergi. One sample was positive for cryptosporidia and none were positive for giardia. This study shows a low level of swimming pool water contamination by microsporidia, cryptosporidia or giardia, demonstrating the efficacy of cleaning filtration and disinfection procedures used in French swimming pools.


http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-3/2/bc784576f28f6ea5b9964074de3cc205

In order to estimate the rate and seasonal variation of Enterocytozoon bieneusi contamination of surface water, sequential samples of water from the River Seine in France were collected during a 1-year period. Each sample (300-600 l) was submitted to sequential filtrations, and the filters were then examined for microsporidia using light microscopy and nested polymerase chain reaction (PCR) for E. bieneusi. Amplified products were hybridized with a E. bieneusi-specific probe. Twenty-five samples of water were analyzed during 1 year. Microscopic examination of stained filters proved unreliable for the identification of spores. Using nested PCR, 16 of 25 specimens were positive (64%). Unexpectedly, E. bieneusi was identified in only one sample by specific hybridization underlining the lack of specificity of ours primers. Nevertheless, using DNA sequence analysis, unknown microsporidia species were identified in eight cases, which had highest scores of homology with Vittaforma corneae or Pleistophora sp. This study shows a low rate of water contamination by E. bieneusi suggesting that the risk of waterborne transmission to humans is limited.

http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-6/2/4b1731aa13d48354e8267cc317ac764b

TnphoA mutagenesis was used to identify adhesins of Aeromonas veronii biovar sobria 3767, a strain isolated from a diarrhoeal stool specimen. Six mutants, from a library of 154, exhibited significantly reduced levels of adhesion to HEp-2 cells. Primers to the terminal regions of TnphoA were used for inverse PCR and the product from one mutant was cloned into pBluescript and partial sequence data obtained. Scanning GenBank and EMBL data bases revealed DNA sequence similarity to the copA gene of Pseudomonas syringae pv. tomato which confers resistance to copper and other heavy metals. The transposon was located within the copA gene and the mutant exhibited a reduced tolerance to copper. Primer walking, using the inverse PCR product as a template, revealed three open reading frames (ORFs) copA, B and C in A. veronii biovar sobria 3767. The predicted amino acid sequences of ORFs A and B had significant homology (55 and 34% respectively) to the copA and B proteins of P. syringae. No amino acid or DNA sequence homology existed between ORF C of strain 3767 and any other gene in the data bases scanned. Further analysis of the nucleotide sequence failed to reveal the presence of typical copper regulatory genes within the vicinity of the Aeromonas sequence. The association between copper tolerance and adhesion in A. veronii biovar sobria requires further study.


http://www.sciencedirect.com/science/article/B6T2T-3XK6TGS-2/2/aab5b51fe49971f953db6e3e6a1ebc1e

Besides group A (GAS), Lancefield group C [beta]-haemolytic streptococci (GCS) have been implicated as a causative agent in outbreaks of purulent pharyngitis. In this study we have investigated a class CI M protein of a Streptococcus dysgalactiae human wound isolate designated MC. MC shares similar properties with M proteins of GAS. It contributes to the virulence of the investigated GCS strain as revealed by in vivo phagocytosis in chicken embryos. Further, MC showed multiple binding to the human plasma proteins fibrinogen, albumin, plasminogen, IgA and all subclasses of IgG. Until now, an M protein, especially from a group C strain, with such a multiple binding behaviour has not been described. Immunoblot experiments with 150 patient sera, having a rheumatoid factor titre >1:256, revealed that 26% of these sera showed serological cross-reactivity between a 68-kDa cartilage protein and the N-terminal part of MC. Only 8% of the sera of healthy patients showed this property. In additional, MC also cross-reacted with antibodies recognising epidermal keratins. The cross-reacting 68-kDa protein from cartilage was different from human serum albumin, but was recognised with anti-vimentin immune serum. The MC was cloned and the gene sequenced. By using PCR, recombinant gene fragments encoding characteristic peptide fragments of MC were expressed in Escherichia coli. The peptides were used to map the binding sites for plasma proteins and to locate the cross-reacting epitopes on the MC molecule. In consequence, sequence alignments revealed that MC shared homologous regions with vimentin and different keratins. Our data, obtained with MC, suggest that not only infections with GAS but also infections with GCS and possibly GGS (the latter species can also produce class CI M-like proteins) may be responsible for the formation of streptococcal-associated sequel diseases.

http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-5/2/31af0f8a6480395605681192a235a513

Twenty-five strains of Plesiomonas shigelloides isolated from aquatic environment, 10 strains from human cases of diarrhoea and five strains from animals were identified by the polymerase chain reaction technique based on 23S rRNA gene. For this purpose, two primers targeted against part of the 5' half of the 23S rRNA gene of P. shigelloides (Escherichia coli number C-912, G-1195; Plesiomonas number C-906, G-1189) were designed. Results from our study indicated that this method might serve as a tool for a rapid and sensitive identification of P. shigelloides from different environmental and clinical sources.


http://www.sciencedirect.com/science/article/B6T2T-405KDGT-D/2/96d5ea12798ad7c921e9405037ce089c

In a period where the proportion of culture confirmed cases in the UK has been steadily declining, diagnosis by PCR has been used to increase the number of confirmed cases and provide additional epidemiological data. This report presents a comparative evaluation of the fluorogenic probe-based 5' exonuclease assay (Taqman) using the Perkin-Elmer Applied Biosystems automated sequence detection system 7700 with previously reported polymerase chain reaction enzyme-linked immunosorbent (PCR ELISA) assays for the detection of meningococcal DNA in CSF, plasma and serum samples. Taqman assays developed were based on the detection of a meningococcal capsular transfer gene (ctrA), the insertion sequence IS1106 and the sialytransferase gene (siaD) for serogroup B and C determination and compared with similar assays in a PCR ELISA format. The Taqman ctrA assay was specific for Neisseria meningitidis, however the IS1106 assay gave false positive reactions with a number of non-meningococcal isolates. Sensitivity of the Taqman ctrA, IS1106 and siaD assays testing samples from culture-confirmed cases were 64, 69 and 50%, respectively, compared with 26, 67 and 43% for the corresponding PCR ELISA assays. Improvements to the DNA extraction procedure has increased the sensitivity to 93 and 91% for the TaqMan(TM) ctrA and siaD assays, respectively, compared to culture confirmed cases. Since the introduction of Taqman PCR a 56% increase in laboratory confirmed cases of meningococcal disease has been observed compared to culture only confirmed cases. The developed Taqman assays for the diagnosis of meningococcal disease enables a high throughput, rapid turnaround of samples with considerable reduced risk of contamination.


http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-8/2/76bac56992f4b4b8d5fcca07ba0c046c
Fusobacterium nucleatum is known to adhere to human polymorphonuclear neutrophils (PMNs) and cause them to aggregate. In this study, we isolated a spontaneously occurring aggregation defective (AGG-) mutant and this mutant will be used for future study of the interactions between this bacterium and human PMN. Genomic DNA fingerprinting by random-primed polymerase chain reaction method revealed a difference between the parent strain and the AGG- mutant. This mutant also showed an altered phenotype in both microbicidal and phagocytic assays, suggesting that the bacterial factor involved in the aggregation may also be very important for the phagocytosis and, subsequently, the killing by human PMNs. Further study of this mutant may help to clarify the molecular mechanisms of the interaction between this pathogen and human PMNs.


http://www.sciencedirect.com/science/article/B6T2T-4BRSXJ6-1/2/4b6a36f994e043d0cab8cf73ebfc586c

In many animal species different intestinal Helicobacter species have been described and a few species are associated with intestinal infection. In humans, the only member of the Helicobacter family which is well described in literature is Helicobacter pylori. No other Helicobacter-associated diseases have definitely been shown in humans. We developed a sensitive quantitative PCR to investigate whether Helicobacter species DNA can be detected in the human gastrointestinal tract. We tested gastric biopsies (including biopsies from H. pylori positive persons), intestinal mucosal biopsies and fecal samples from healthy persons, and intestinal mucosal biopsies from patients with inflammatory bowel disease (IBD) for the presence of Helicobacter species. All gastric biopsies, positive for H. pylori by culture, were also positive in our newly developed PCR. No Helicobacter species were found in the mucosal biopsies from patients with IBD (n=56) nor from healthy controls (n=25). All fecal samples were negative. Our study suggests that Helicobacter species, other than H. pylori, are not present in the normal human gastrointestinal flora and our results do not support a role of Helicobacter species in IBD.


http://www.sciencedirect.com/science/article/B6T2T-3Y6RF1B-6/2/acc6359e29aea3fd0a13fe05bf9537

The polymerase chain reaction (PCR) method has been employed to amplify a chlamydial genome encoding four variable segments of the major outer membrane protein and genotyping of different Chlamydia trachomatis serovars was successfully achieved by means of restriction fragment length polymorphism (RFLP) analysis and sequencing of amplified DNA. These methods were applied to identify the serotypes of C. trachomatis in endocervical specimens obtained from asymptomatic pregnant Japanese women at 28-30 weeks of gestation. Among the 218 specimens, 207 were serotyped 43 (19.3%) as serovar D, 53 (24.3%) as E, 24 (11.0%) as F, 39 (17.9%) as G, 15 (6.9%) as H, 15 (6.9%) as I, five (2.3%) as J, nine (4.1%) as K and four (1.8%) as mixed. Among the 11 unclassified strains by RFLP, six (2.8%) were identified as serovar B variants and five (2.3%) were identified as D/IC-Cal-8. It was suggested that variants of endemic trachoma serovars also have affinity for the urogenital tract of Japanese pregnant women.

http://www.sciencedirect.com/science/article/B6T2T-3S1PY2B-6/2/59f57d49a729dcb5d3c05c83d2

Using defined rfb mutants, defective in the biosynthesis of the O-antigen of the lipopolysaccharide (LPS), and monoclonal antibodies (MAbs) to the A, B and C LPS antigens, we have examined the distribution of the antigens and the effects of their loss. By immunogold electron microscopy, it has been possible to determine the relative amounts of the A, B and C antigens on Inaba and Ogawa cells, confirming previous studies based upon bacterial agglutination and hemagglutination inhibitions. These antigens are absent from rfb:Tn mutants selected as resistant to phages which have been shown to use the O-antigen as their receptor. These mutants were severely attenuated as measured by both LD50 and their ability to compete with the wild-type parents when analyzed in the infant mouse cholera model. These mutants were unchanged in the export of choler toxin or other secreted proteins but revealed an altered outer membrane protein profile. The competition defect suggested an effect on TCP (toxin-coregulated pilus). An analysis of the rfb:Tn mutants revealed that they were unable to assemble TCP on their surface, but the major subunit, TcpA, could be found as an intracellular pool. These mutants could be complemented back to wild-type using the cloned rfb region, implying that functional TCP assembly is dependent upon an intact LPS.


http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-2/2/b1002971eec533e02d18630cc36da211

We previously demonstrated that interleukin (IL)-12 protected mice against fatal pulmonary infection with a highly virulent strain of Cryptococcus neoformans, which correlated well with the production of interferon (IFN)-[gamma] as well as IL-18 in the primary infected site. In the present study, we examined the role of endogenously synthesized IL-18 in IL-12-induced host resistance to this pathogen. There was little or no production of IFN-[gamma] and IL-18 both at mRNA and protein levels in lungs of mice infected with C. neoformans, while treatment with IL-12 induced a marked production of these cytokines. Caspase-1 mRNA was expressed in infected mice even without IL-12 treatment. Administration of neutralizing anti-IFN-[gamma] monoclonal antibody (mAb) clearly inhibited production of IFN-[gamma] and IL-18 induced by IL-12, while control IgG did not show such an effect. However, administration of IFN-[gamma] did not induce the production of both cytokines in infected mice, although tumor necrosis factor (TNF)-[alpha] and IFN-[gamma]-inducible protein (IP)-10 were synthesized by the same treatment. Finally, neutralizing anti-IL-18 antibody (Ab) significantly interfered with the production of IFN-[gamma] and elimination of the microorganism from the lung induced by IL-12 treatment. Furthermore, both IFN-[gamma] synthesis and host protection caused by IL-12 were profoundly diminished in IL-18 gene-disrupted mice. Considered collectively, our results indicated that host protection against C. neoformans induced by IL-12 involved endogenously synthesized IL-18 and that the production of IL-18 was mediated at least in part by endogenous IFN-[gamma].
We examined the mechanisms involved in the development of lung lesions after infection with Cryptococcus neoformans by comparing the histopathological findings and chemokine responses in the lungs of mice infected with C. neoformans and assessed the effect of interleukin (IL) 12 which protects mice from lethal infection. In mice infected intratracheally with a highly virulent strain of C. neoformans, the yeast cells multiplied quickly in the alveolar spaces but only a poor cellular inflammatory response was observed throughout the course of infection. Very little or no production of chemokines, including MCP-1, RANTES, MIP-1[alpha], MIP-1[beta] and IP-10, was detected at the mRNA level using RT-PCR as well as at a protein level in MCP-1, RANTES and MIP-1[alpha]. In contrast, intraperitoneal administration of IL-12 induced the synthesis of these chemokines and a marked cellular inflammatory response involving histiocytes and lymphocytes in infected mice. Our findings were confirmed by flow cytometry of intraparenchymal leukocytes obtained from lung homogenates which showed IL-12-induced accumulation of inflammatory cells consisting mostly of macrophages and CD4+ [alpha][beta] T cells. On the other hand, C-X-C chemokines including MIP-2 and KC, which attract neutrophils, were produced in infected and PBS-treated mice but treatment with IL-12 showed a marginal effect on their level, and neutrophil accumulation was similar in PBS- and IL-12-treated mice infected with C. neoformans. Our results demonstrate a close correlation between chemokine levels and development of lung lesions, and suggest that the induction of chemokine synthesis may be one of the mechanisms of IL-12-induced protection against cryptococcal infection.

Two divergently transcribed open reading frames: cpsX and cpsY separated by a common regulatory region was identified upstream of the cpsA-D genes involved in polysaccharide capsule biosynthesis in group B streptococci (GBS). We suggest that these genes are involved in the regulation of capsule expression in GBS, since the CpsX protein shares sequence similarities with LytR of Bacillus subtilis, an attenuator of transcription while CpsY has similarity to a wide variety of members of the LysR family of transcriptional regulators. No deletions, insertions, DNA rearrangements, or apparent differences were discovered in the postulated regulatory genes when the gene region was compared in GBS with different capsule phenotypes. Thus, other yet unidentified gene loci may control capsule phase variation in GBS.

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http://www.sciencedirect.com/science/article/B6T2T-3TMXY36-3/2/3e9a3f9571085280fe98567790736eae
S and F1C fimbrial adhesins often expressed by uropathogenic Escherichia coli are genetically homologous. A multiply primed polymerase chain reaction (PCR) was developed for discriminating the S (sfa) and F1C (foc) fimbrial operons. A total of 270 uropathogenic E. coli strains and 80 fecal isolates were examined. PCR specifically detected the sfa and foc alleles in 105 (93%) of 113 sfa/foc+ strains by DNA hybridization. Furthermore, 87% of sfa+ uropathogenic E. coli simultaneously possessed the genes encoding the class III P fimbrial adhesin (prsGJ96), [alpha]-hemolysin and cytotoxic necrotizing factor 1. Statistical analysis showed the class II P fimbrial adhesin (papGIA2) and F1C fimbria to be associated with high relative virulence in pyelonephritis and cystitis, respectively. The multiply primed PCR developed should be useful for assessing the contribution of the S and F1C fimbriae in the pathogenesis of urinary tract infections.


http://www.sciencedirect.com/science/article/B6T2T-3W32484-J/2/0196da240bd9b42c5e5ac58ddf42d8b6

We measured urinary endotoxin, IL-6 and IL-8 levels in 23 patients with gram-negative urosepsis. The endotoxin and cytokine levels showed a 100-1000 fold range. No correlation was found between levels of urinary endotoxin, and IL-6 or IL-8 levels. In all cases bacterial numbers were >= 105 CFU ml-1 urine. The endotoxin content of the isolated microorganisms neither correlated with the urinary cytokine levels, nor with IL-6 and IL-8 levels obtained in vitro when 103 log-phase CFU of each of the bacteria were incubated with heparinized whole blood of three healthy donors. Neither the haemolysin phenotype of the bacteria, nor the presence of the P-pili gene was correlated with the cytokine response in vivo or in vitro. Other factors than known bacterial virulence factors apparently contribute to the wide variation in urinary cytokine levels in urinary tract infection.


http://www.sciencedirect.com/science/article/B6T2T-4CYPVKP-1/2/f1c106edac1f2b8c3da53b7e016fd1e3

The aim of the study was to assess the quantitative and qualitative differences of the gut microbiota in infants. We evaluated gut microbiota at the age of 6 months in 32 infants who were either exclusively breast-fed, formula-fed, nursed by a formula supplemented with prebiotics (a mixture of fructo- and galacto-oligosaccharides) or breast-fed by mothers who had been given probiotics. The Bifidobacterium, Bacteroides, Clostridium and Lactobacillus/Enterococcus microbiota were assessed by the fluorescence in situ hybridization, and Bifidobacterium species were further characterized by PCR. Total number of bifidobacteria was lower among the formula-fed group than in other groups (P = 0.044). Total amounts of the other bacteria were comparable between the groups. The specific Bifidobacterium microbiota composition of the breast-fed infants was achieved in infants receiving prebiotic supplemented formula. This would suggest that early gut Bifidobacterium microbiota can be modified by special diets up to the age of 6 months.

http://www.sciencedirect.com/science/article/B6T2T-3SCJ0T1-7/2/8cec095b460538d33232f9ae0737c15a

Haemophilus influenzae and Streptococcus pneumoniae are often the cause of serious diseases such as meningitis. We designed a nested PCR assay to identify these pathogens from cerebrospinal fluid samples. The first-step PCR was able to detect eubacterial rRNA genes with a unified set of universal primers. In the second-step PCR, the identification primers, HI I and II and SP I and II, could detect H. influenzae and S. pneumoniae respectively through amplification of the rRNA spacer between the 16S and 23S rRNA genes. We suggest that the two-step PCR assay can be used as a novel method for the immediate and retrospective diagnosis of bacterial meningitis caused by H. influenzae and S. pneumoniae.


http://www.sciencedirect.com/science/article/B6T2T-44JD8N7-1/2/2d1281ebfc2e105f1b0032ae19a6d4d9

A repetitive DNA motif was used as a marker to identify novel genes in the mucosal pathogen Moraxella catarrhalis. There is a high prevalence of such repetitive motifs in virulence genes that display phase variable expression. Two repeat containing loci were identified using a digoxigenin-labelled 5'-((CAAC)6)-3' oligonucleotide probe. The repeats are located in the methylase components of two distinct type III restriction-modification (R-M) systems. We suggest that the phase variable nature of these R-M systems indicates that they have an important role in the biology of M. catarrhalis.


http://www.sciencedirect.com/science/article/B6T2T-3S6TYBD-C/2/445f1dceff9e5583544c58e13df1a224

Two Swedish isolates of Coxiella burnetii and the two prototype strains of the species, Nine Mile and Priscilla, were characterized with regard to their multiplication and cytopathic effect on BGM cells and by PCR-based amplification of repetitive element DNA and the C. burnetii-specific plasmids QpH1 and QpRS. Moreover, 1330 bp of each 16S rRNA gene were sequence-determined. All four strains multiplied at virtually the same rate and displayed the same type of vacuoles in the BGM cells. Genetic homogeneity was observed inasmuch as the 16S rDNA sequences were identical and the strains showed identical PCR amplification patterns using primers specific to enterobacterial repetitive intragenic consensus DNA sequences. The two Swedish strains and the Priscilla strain also showed identical patterns after PCR amplification of repetitive extragenic palindromic DNA sequences, whereas the Nine Mile strain demonstrated a similar, but not identical pattern. Thus, the investigated strains demonstrated very similar phenotypic and genotypic characteristics. This finding is discussed in view of the very rare occurrence of domestic Q fever in Sweden.

http://www.sciencedirect.com/science/article/B6T2T-463NNRG-1D/2/00faa2e15bec91717ab0fdb533c16ae6

Genetic diversity of the streptokinase gene (sk) from 36 strains of *S. equisimilis* and 54 strains of group G streptococci was examined. The strains were isolated from patients with various streptococcal disease manifestations and healthy carriers. The region of the gene that corresponds to amino acid residues 174-244, was PCR amplified. The amplified product was subjected to *MluI*, *PvuII*, *DraI* and *DdeI* digestion. Based on the restriction enzyme digestion patterns nine sk alleles were recognized. There was no correlation between the various sk gene alleles and streptococcal disease manifestations. Three of the nine sk gene alleles, sk4, sk7, and sk8, were detected earlier among group A streptococci. The other six alleles were unique to *S. equisimilis* and group G streptococci. The most common alleles were sk5, found in 21/90 (23%) and sk10 detected in 43/90 (47%) of the strains. Alleles sk1 and sk2, the most frequent among group A streptococci, were not found among the strains in the present investigation. Thus, it appears that the sk gene has been evolving in line with other species distinguishing features of the streptococci.


http://www.sciencedirect.com/science/article/B6T2T-3WG32M9-C/2/c941b3ddcd90610501d00472670fbdf0f3

A polymerase chain reaction assay (PCR) for the diagnosis of Helicobacter pylori in human gastric biopsies was developed. To prevent false-negative results while performing PCR on human tissues, an internal control is necessary. Primer set ACT1-ACT2 which specifically amplifies a 542-bp fragment of the 16S rRNA gene of *H. pylori* was used. dUTP and hot-start were used to prevent false-positives from carryover of previous products and avoid non-specific extension products. A competitive internal control DNA fragment was constructed to detect the presence of inhibitors. Biopsies from 101 unselected patients with gastric symptoms were tested. PCR results were compared with results from microscopy of histological sections and conventional culturing for *H. pylori*. Forty-two percent of the biopsies were found to contain compounds inhibiting the PCR. The addition of the internal control assures the performance of the PCR assay and is an important quality control parameter.


http://www.sciencedirect.com/science/article/B6T2T-41F73GN-R/2/ca49eb7c1dcc2ab605ff26f532b1f90a

A primer-set was designed for specific detection of genes that encode for 16S rRNA of Helicobacter pylori, using direct polymerase chain reaction (PCR). The primers were selected in the hypervariable regions, derived from a complete small subunit 16S rRNA sequence of the
reference strain H. pylori CCUG 17874. The primer-set amplified a 537 base pair (bp) sequence specifically from chromosomal H. pylori DNA. Amplification of purified chromosomal H. pylori DNA was achieved at concentrations as low as 1 femto gram (fg), equivalent to 5 bacteria. Furthermore, as few as 1 lysed H. pylori cell was detected by this PCR technique. The specificity of the primers was 100%, since purified chromosomal DNA was detected from all 32 various H. pylori isolates, whereas no other bacteria species were detected, whether related to Helicobacter or not. The 16S rDNA primers successfully detected H. pylori in antral biopsy specimens collected from infected patients.


http://www.sciencedirect.com/science/article/B6T2T-3RHN702-7/2/62cbf3584c8f34a0f55f52294d702e60

Prevalence of Mycoplasma genitalium in humans is still not clear. We have developed a sensitive and specific serological assay for M. genitalium using lipid-associated membrane proteins (LAMPs) as antigens. Antibodies to LAMPs from M. genitalium showed little cross-reactivity to LAMPs from antigenically similar M. pneumoniae. For validity testing, urines from 104 patients were tested by PCR for M. genitalium. All 15 PCR+ patients had M. genitalium-LAMPs antibodies. Moreover, none of 64 antibody-negative patients were PCR+. Serological study of 1800 patients of various diseased groups and healthy blood donors showed M. genitalium was primarily a sexually transmitted microbe that infected patients with AIDS (44.0%), intravenous drugs users with or without HIV infection (42.5%), and also HIV- patients attending STD clinics (42.6%). Only 5.5% HIV- healthy blood donors and 1.3% HIV+ hemophiliacs tested positive. M. genitalium has been associated with acute non-gonococcal urethritis in male patients. However, many sexually active men and women appear to be chronically infected or colonized by the microbe without apparent clinical symptoms and may continue to transmit the organism through sexual contacts.


http://www.sciencedirect.com/science/article/B6T2T-4SBNC1-1/2/c0228c25f43d9b9ae99bcbf41938d001

Campylobacter jejuni is a leading human food-borne pathogen. The rapid and sensitive detection of C. jejuni is necessary for the maintenance of a safe food/water supply. In this article, we present a real-time polymerase chain reaction (PCR) assay for quantitative detection of C. jejuni in naturally contaminated poultry, milk and environmental samples without an enrichment step. The whole assay can be completed in 60 min with a detection limit of approximately 1 CFU. The standard curve correlation coefficient for the threshold cycle versus the copy number of initial C. jejuni cells was 0.988. To test the PCR system, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of C. jejuni. 30.6% (92/300) of chicken meat samples, 27.3% (82/300) of milk samples, and 13.6% (41/300) of water samples tested positive for C. jejuni. This result indicated that the real-time PCR assay provides a specific, sensitive and rapid method for quantitative detection of C. jejuni. Moreover, it is concluded that retail chicken meat, raw milk and environmental water are commonly contaminated with C. jejuni and could serve as a potential risk for consumers in eastern China, especially if proper hygienic and cooking conditions are not maintained.

http://www.sciencedirect.com/science/article/B6T2T-4BKDRSC-1/2/683fc1eef00e748933a8788c981e409

Phenolic glycolipid-I (PGL-I), a Mycobacterium leprae-specific antigen, has been widely used for the serodiagnosis of leprosy and has been implicated in the pathogenesis of leprosy. In an effort to produce an alternate antigen of PGL-I, the mimotope peptides of PGL-I, W(T/R)LGPY(V/M), were obtained using a monoclonal antibody, III603.8, specific to PGL-I by a phage library. The biotin-labeled predominant mimotope peptide of PGLP1, WTLGPYV, bound to III603.8 in a dose-dependent manner in an immunoassay. However, PGLP1 did not bind to anti-PGL-I antibodies in the serum samples from leprosy patients that were reactive to PGL-I. Although the mimotope peptide of WTLGPYV was not effective as an alternate antigen of PGL-I for the serodiagnosis of leprosy, it would be of interest to know how the mimotope peptides mimic the role of PGL-I antigen in the pathogenesis of M. leprae infection.


http://www.sciencedirect.com/science/article/B6T2T-46VJGKR-1/2/89e30a15479a581872e32a6d617cc82b

Neisseria meningitidis shows great variation in expression of structurally different lipooligosaccharides (LOS) on its cell surface. To better understand the LOS diversity that may occur within an individual strain, a group C wild-type strain, BB305-Tr4, and two stable isogenic LOS variants, Tr5 and Tr7, were selected for this study. SDS-PAGE analysis showed a size reduction of Tr5 and Tr7 LOS compared to that of Tr4. Immunoblotting showed that parental Tr4 LOS reacted with L1, L2 and L3,7 antibodies, variant Tr5 LOS with L1 and L6 antibodies, while Tr7 LOS was non-typeable. Genetic analysis showed that the gene organization at the lgt-1 locus in the three strains was lgtZ,C,A,B,H4 in Tr4, lgtZ,C,A,H4 in Tr5 and lgtZ,C,A,H9 in Tr7. The genetic differences in the three strains were consistent with their phenotypic changes. Sequence comparison revealed two independent recombination events. The first was the recombination of repeated DNA fragments in the flanking regions to delete lgtB in Tr5. The second was the recombination of a fragment of two genes, lgtB and lgtH4, to create an inactive lgtH9 allele with a mosaic structure in Tr7. These findings suggest that besides phase variation, homologous recombination can contribute to the genetic diversity of the lgt locus and to the generation of LOS variation in N. meningitidis.

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Southern blot analysis of DNA from an iron-oxidising moderate thermophile NMW-6 and from Thiothrix ferrooxidans strain TF1-35 demonstrated sequences homologous to the RuBisCO LSU gene of Synechococcus. DNA fragments (457 bp) encoding part of the RuBisCO LSU gene (amino acids 73-200) were amplified from the genomic DNA of Thiothrix ferrooxidans and the moderate thermophile NMW-6 using the polymerase chain reaction (PCR) technique (Saiki et al. (1985) Science 233, 1350-1354). A comparison with the LSU sequences from T. ferrooxidans, Alcaligenes eutrophus, Chromatium vinosum, Synechococcus and Spinacea oleracea, which all have RuBisCOs with a hexadecameric structure, showed that the RuBisCO LSU gene sequence from NMW-6 appeared to be most closely related to that of the hydrogen bacterium A. eutrophus which showed 71.9% homology at the amino acid level. Despite its physiological similarity, T. ferrooxidans showed only 64.1% homology to the amino acid sequence from NMW-6 and had the lowest DNA homology (60.9%) of the hexadecameric type RuBisCOs. In the region sequenced, T. ferrooxidans and the RuBisCOs of the phototrophs C. vinosum, Synechococcus and S. oleracea, had 17 residues that were completely conserved which were substituted in both NMW-6 and A. eutrophus, 11 of these being identical substitutions. Comparison of the nucleotide and derived amino acid sequences of the RuBisCO LSU fragment from T. ferrooxidans with other RuBisCO sequences indicated a closer relationship to the hexadecameric type LSU genes of photosynthetic origin than to that of A. eutrophus. The T. ferrooxidans amino acid sequence showed 93.8%, 78.9% and 77.3% homology, respectively, to the C. vinosum, Synechococcus and S. oleracea (spinach) sequences but only 56.2% to A. eutrophus. The DNA sequence from Rhodospirillum rubrum, which has the atypical large subunit dimer RuBisCO structure with no small subunit, showed 39.2% and 42.7% homology, respectively, with the sequences of NMW-6 and T. ferrooxidans, and 25.0% and 29.7% amino acid homology, indicating that the DNA homology was substantially random in nature. PCR fragments (126 bp) that overlapped the last 15 codons of the fragments above were also amplified and sequenced. They showed incomplete homology with the larger fragments, supporting evidence obtained from Southern hybridizations that T. ferrooxidans and the moderate thermophile NMW-6 have multiple copies of RuBisCO LSU genes.


DMS (dimethyl sulfide) is an important beer flavor compound which is derived either from the beer wort production process or via the brewing yeast metabolism. We investigated the contribution of yeast MXR1 gene activity to the final beer DMS content. The MXR1-CA gene from Saccharomyces carlsbergensis (synonym of Saccharomyces pastorianus) lager brewing yeast was isolated and sequenced, and found to be 88% identical with Saccharomyces cerevisiae MXR1. Inactive deletion alleles of both genes were substituted for their functional counterparts in S. carlsbergensis. Such yeasts fermented well and did not form DMS from dimethyl sulfoxide. Overexpression in brewing yeast of MXR1 from non-native promoters with various strengths and transcription profiles resulted in an enhanced and correlated DMS production. The promoters of
MXR1 and MXR1-CA contain conserved Met31p/Met32p binding sites, and in accordance with this were found to be co-regulated with the genes of the sulfur assimilation pathway. In addition, conserved YRE-like DNA sequences are present in these promoters, indicating that Yap1p may also take part in the control of these genes.


http://www.sciencedirect.com/science/article/B6W8C-44JJF6D-1/2/52a6b88f02d2a3d31171a2a10dd7e8c7

The allopolyploid yeast Saccharomyces carlsbergensis appears to be a relatively newly formed species hybrid, and therefore constitutes a good model for studying early steps in hybrid speciation. Using reverse transcription-coupled polymerase chain reaction to monitor derepression of the S. carlsbergensis homologues of the sulfur assimilation genes MET14 and MET2, we found that both homologues of these genes are regulated in the same pathway-specific manner, but surprisingly, with different kinetics, as the genes derived from one of the parent species (the non-Saccharomyces cerevisiae-like) are alleviated from repression much faster than the genes from the other parent (the S. cerevisiae-like). This probably reflects differing physiological adaptation of the parent species, and the finding may contribute to the general understanding of hybrid speciation.


http://www.sciencedirect.com/science/article/B6W8C-468CC2G-1/2/28e6cd4cd0a91aa37b2df9da37ea87d4

Cryptococcus neoformans var. gattii (serotypes B and C) is a human pathogen, ecologically, biochemically, clinically and genetically different from C. neoformans var. grubii (serotype A) and C. neoformans var. neoformans (serotype D). The phospholipase B (PLB1) gene from serotypes B and C was isolated and characterised. It resembled the serotype A and D genes, with an overall sequence homology of more than 85%. The respective open reading frames were 2236 bp (serotype B) and 2239 bp (serotype C) in length. Each contained six introns and encoded a 68-kDa protein destined for secretion. PLB1 was located on the second smallest chromosome in both serotypes. Gene expression, measured as mRNA, was not regulated by temperature, pH or exogenous nutrients.

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Objective: To evaluate the level of 17[beta]-hydroxysteroid dehydrogenase (17[beta]-HSD) type 1, 2, and 3 transcripts in a Sertoli-Leydig cell tumor, adjacent theca lutein ovarian tissue, and normal control ovarian stromal tissue. Setting: An academic medical center. Design: Total RNA was extracted from formalin-fixed, paraffin-embedded tissue slides and used for reverse transcription-polymerase chain reaction (RT-PCR) with primers specific for 17[beta]-HSD types 1, 2, or 3. The PCR products were subjected to Southern hybridization with 5’ [32p] end-labeled internal primers for each type of the isozymes. Patient(s): A 35-year-old woman with a Sertoli-Leydig cell tumor on her right ovary. The tumor and the right ovary were surgically removed. Control ovarian stromal tissue was obtained from a woman undergoing hysterectomy for uterine leiomyomata. Result(s): In the control ovarian stromal tissue, the transcripts for the type 1 and type 2 isoforms were the predominant transcripts detected. In the Sertoli-Leydig cell tumor, the transcript for the type 3 isoform was the predominant transcript detected. Ovarian tissue from the same ovary as the Sertoli-Leydig cell tumor (diagnosed as theca lutein cysts on histologic examination) expressed the type 2 and 3 transcripts. Conclusion(s): The 17[beta]-HSD type 3 isoform efficiently converts androstenedione to T and is the predominant HSD isoform in the testis. In hyperandrogenism caused by a Sertoli-Leydig cell tumor, both the tumor tissue and nontumor tissue from the same ovary expressed the "testicular" form of the 17[beta]-HSD.


Objective: To examine changes in oviductin mRNA expression in oviductal mucosal tissue from fertile women throughout an ovulatory cycle. Design: Semiquantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis of oviductin mRNA. Setting: University-based obstetrics and gynecology department. Subject(s): Twenty women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids. Intervention(s): The mucosal layer was isolated from the oviduct tissue, and semiquantitative RT-PCR was performed. Main Outcome Measure(s): The relationship between serum estradiol, luteinizing hormone, and progesterone concentrations and the expression of oviductin mRNA. Result(s): There was a significant positive correlation between serum estradiol and luteinizing hormone concentrations and oviductin mRNA expression. There was a significant inverse correlation between serum progesterone concentrations and oviductin mRNA expression. Conclusion(s): Little is known about the regulation of human oviductin. This study was the first to examine the relationship between oviductin mRNA expression and serum estradiol and luteinizing hormone and progesterone concentrations in fertile women. Estradiol and luteinizing hormone both have a stimulatory effect on oviductin mRNA in humans, however, it is difficult to determine whether the effects are independent of one another, as the luteinizing hormone surge is dependent on the estradiol increase. Progesterone shows a clear inhibitory effect on oviductin mRNA.

Objective: To determine whether oviduct mucosal cell culture with exogenous 17[beta] E2 supports the continued production of oviductin, a putative embryotrophic protein.

Design: Semiquantitative reverse-transcriptase polymerase chain reaction analysis of oviductin mRNA expression after oviduct mucosal cell culture in the presence of 17[beta] E2. Three different culture systems were studied to investigate the response to E2.

Setting: University-based obstetrics and gynecology department.

Subjects: Oviduct tissue was obtained from 18 women undergoing laparoscopy for benign gynecologic conditions.

Intervention(s): The mucosal layer was isolated from the oviduct tissue and exposed to three different culture systems, which contained various concentrations of 17[beta] E2, or vehicle-only control.

Main outcome measure(s): The relationship between exposure to 17[beta] E2 and expression of oviductin messenger (m)RNA by cultured oviduct mucosal cells.

Result(s): There was a significant increase in oviductin mRNA expression after the addition of 17[beta] E2 to the culture system in which the in vivo cell-to-cell and cell-to-basement-membrane contacts of the oviduct had been maintained.

Conclusion(s): Estradiol failed to alter oviductin mRNA expression in oviduct mucosal cells cultured under conditions in which the ciliated mucosal cell phenotype plus the cell-to-cell and cell-to-basement-membrane contacts of the oviduct were lost. However, with a culture system that maintained the cell architecture, E2 initiated and significantly increased oviductin mRNA expression.


http://www.sciencedirect.com/science/article/B6T6K-49J881K-6/2/2c2cd0cca6cc4467da03b361f02bc1b

Objective: To determine whether oviduct mucosal cell culture with exogenous hCG or 17-[beta] estradiol (E2) supports the continued production of oviductin, a putative embryotrophic protein.

Design: Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of oviductin mRNA expression after oviduct mucosal cell culture in the presence of hCG or 17-[beta] E2.

Setting: University-based Obstetrics and Gynecology Department.

Subject(s): Ten women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids.

Intervention(s): The mucosal layer was isolated from the oviduct tissue, subjected to routine culture conditions with the addition of various concentrations of hCG or 17-[beta] E2 or the equivalent vehicle-only control and semiquantitative RT-PCR performed.

Main outcome measure(s): The relationship between exposure to hCG or 17-[beta] E2 and expression of oviductin mRNA by cultured oviduct mucosal cells.

Result(s): There was a significant increase in oviductin mRNA expression after the addition of hCG to the culture medium but only in samples that had maintained a baseline level of oviductin expression. Addition of 17-[beta] E2 to the culture medium had no significant effect on oviductin mRNA expression.

Conclusion(s): Under standard cell culture conditions, baseline human oviductin mRNA expression is increased by the addition of hCG. This effect is likely to be a secondary or synergistic effect as exogenous hCG failed to restore oviductin mRNA expression in samples where expression was lost after culture. E2 failed to alter oviductin mRNA expression in oviduct mucosal cells cultured under these conditions.


http://www.sciencedirect.com/science/article/B6T6K-456BW2B-X/2/947f144b8ae9910d6074306ccda1a0e2

Objective: To determine whether oviduct mucosal cell culture supports the continued production
of oviductin, a putative embryotrophic protein. Design: Semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of oviductin messenger RNA (mRNA) expression after oviduct mucosal cell culture. Setting: University-based obstetrics and gynecology department. Patient(s): Ten women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids. Intervention(s): The mucosal layer was isolated from the oviduct tissue and subjected to routine culture conditions; semiquantitative RT-PCR was performed. Main Outcome Measure(s): The relationship between duration of cell culture and expression of oviductin mRNA. Result(s): There was a significant reduction in oviductin mRNA expression after 3 days in culture, with a complete loss after 6 days in 70% of the samples and after 12 days in the remaining 30%. Conclusion(s): This is the first study to investigate whether oviductin mRNA continues to be expressed in cultured human oviduct mucosal cells. Our results suggest that oviduct mucosal cells lose their ability to produce oviductin after short-term culture. This method of culture does not appear to be appropriate for a coculture system reliant upon oviductal secretion of oviductin.


http://www.sciencedirect.com/science/article/B6T6K-44T0V32-6/2/f2f6f662cc2a81de5d837a74282503

Objective: To evaluate the association between endometriosis and the p53 polymorphism. Design: Prospective study. Setting: Department of gynecology and genetics in a medical center. Patient(s): Women with and without endometriosis. Intervention(s): Women were categorized as having moderate or severe endometriosis (n = 118) or no endometriosis (n = 140). Main Outcome Measure(s): Polymerase chain reaction was used to detect p53 codon 72 polymorphisms (arginine homozygosity, heterozygosity, and proline homozygosity). Associations between endometriosis and p53 polymorphisms were evaluated. Result(s): The distributions of different p53 polymorphisms differed significantly between groups. The respective proportions of arginine homozygotes, heterozygotes, and proline homozygotes were 10.2%, 66.9%, and 22.9% in the group with endometriosis and 30.7%, 50%, and 19.3% in the group without endometriosis. Conclusion(s): Endometriosis is associated with p53 polymorphism. p53 arginine homozygotes have lower risk for endometriosis. Heterozygotes and proline homozygotes have higher risk for endometriosis.


http://www.sciencedirect.com/science/article/B6T6K-3WS633J-14/2/8df9859637c4597b7bd730517814f1cf

Objective: To describe the molecular, cytogenetic, immunohistochemical, and endocrinologic characteristics of a young 46,XY female with persistent mullerian structures and germ cell tumors in dysgenetic gonads. Design: Descriptive case study. Setting: Mackay Memorial Hospital and National Yang-Ming University, Taipei, Taiwan. Patient(s): A 22-year-old 46,XY female with persistent mullerian structures, a low level of serum testosterone, and no apparent adnexal masses. Intervention(s): Laparoscopic removal of the dysgenetic gonads. Main Outcome Measure(s): Detection of an androgen receptor gene mutation by a semiautomated DNA sequencer, of the chromosomal complement by cytogenetic examination, of placental alkaline phosphatase activity by immunohistochemical analysis, and of neoplasms in dysgenetic gonads by histologic studies. Result(s): A unilateral gonadoblastoma and a contralateral gonadoblastoma...
Molecular genetic analysis of the androgen receptor gene aids in the rapid diagnosis of complete androgen insensitivity irrespective of atypical clinical phenotypes and endocrinologic parameters.


expression. Conclusion(s): Overexpression of CYP17 and CYP11A mRNA in theca cells from polycystic ovaries is explained by polymorphic differences in the gene promoters.


http://www.sciencedirect.com/science/article/B6T6K-3VKJXCV-1S/2/ebd34c78f7337c4f8d4317da588ba4

Objective: To analyze the effect of different concentrations of ofloxacin on sperm kinematic parameters and to determine the embryotoxicity of ofloxacin at physiologic and at 100 x concentrations. Design: Prospective comparative study. Setting: Clinical and academic research environment. Patient(s): Pooled cryopreserved donor sperm (n = 7). Intervention(s): Human sperm were processed through two-layer discontinuous Percoll gradients, and the resultant pellet was resuspended in either HEPES-buffered human tubal fluid medium containing different concentrations of ofloxacin or the control medium. After measuring the kinematic parameters, the percentages of apoptosis and viability were obtained. Next, the sperm DNA was extracted and polymerase chain reaction of P-globin gene was performed followed by denaturing gradient gel electrophoresis. Mouse embryos recovered at the one-cell pronuclear or zygote stages were cultured in the presence or absence of ofloxacin up to the hatched blastocyst stage and differences in development were recorded. Main Outcome Measure(s): Sperm kinematic parameters, sperm P-globin gene, and number of embryos reaching the hatched blastocyst stage. Result(s): The number of embryos exposed to control and physiologic ofloxacin concentrations showed comparable excellent growth. However, the 100 x concentration significantly arrested development. Rates of sperm viability and apoptosis measured 48 hours after exposure to the above concentrations were not different from controls. No differences were noted in the sperm kinematic parameters of sperm exposed to ofloxacin concentrations (1 X, 10 x, and 100X) or control medium after 0, 4, and 48 hours of incubation. Denaturing gradient gel electrophoresis of [beta]-globin genes from DNA exposed to varying ofloxacin concentrations failed to show any point mutations. Conclusion(s): Ofloxacin was embryotoxic at pharmacologic concentrations (100 x). At physiologic or higher concentrations, ofloxacin appears to be safe and does not affect sperm kinematic parameters when compared with controls. This may indicate that sperm motility parameters alone cannot be relied on to evaluate the effects of drugs on fertility and that in vitro embryologic studies are essential. Ofloxacin at any concentration did not alter the rates of sperm apoptosis or viability. Ofloxacin does not appear to be mutagenic as evidenced by the [beta]-globin gene analysis.


http://www.sciencedirect.com/science/article/B6T6K-4BY331V-1B/2/60049cc3143f1dab9a48ce0cbb9b9050

Objective: To find the underlying defect in a case of primary FSH deficiency and to estimate the beneficial effect of FSH treatment. Design: Case report. Setting: University hospital fertility clinic. Patient(s): Normal, healthy, 37-year-old male patient with severe oligoteratozoospermia. Intervention(s): Levels of FSH, LH, LHRH provocation test, karyotyping, genomic analysis on the Y-chromosomal AZF region and sequencing of the FSHB gene, FSH treatment. Main outcome measure(s): We compiled detailed clinical and molecular data on four pregnancies. We compare this case with a similar case published recently. Result(s): There were detectable but very low FSH levels after LHRH provocation; the LH response was not entirely normal, and no genomic abnormalities were found in the FSHB gene. The FSH treatment
resulted in four pregnancies, two of which ended in abortion; the other two resulted in the birth of two healthy children. Both our case and the published case had detectable but abnormally low FSH levels on some occasions, but normal or highly normal inhibin B levels that differed from the expected low levels. Both patients had a normal male phenotype and no detectable mutation in the FSHB gene. The published case differed from our patient in that the published case was azoospermic whereas ours was extremely oligoteratozoospermic. The beneficial effect of FSH treatment was only shown in our patient.

Conclusion(s) The published case and ours may have a common, as yet unidentified, underlying defect. The dramatic and immediate effect of FSH treatment on our patient's fertility was clearly demonstrated.


http://www.sciencedirect.com/science/article/B6T6K-4FM9D09-9/2/3cda3b300cf292ec5ca21baa6f9380f

Objective To investigate the association of endometriosis with estrogen receptor alpha (ER[alpha]) and cytochrome P450c17[alpha] (CYP17) gene polymorphisms in light of the fact that estrogen plays a role in the pathogenesis of endometriosis and the CYP17 enzyme is involved with estrogen biosynthesis.

Design Prospective study.

Setting Genetics and gynecology units.

Patient(s) All patients were divided into two groups: group 1, women with endometriosis (n = 119); group 2, normal controls (n = 108).

Intervention(s) A dinucleotide (thymine-adenine [TA]) repeat polymorphism lying upstream of the ER[alpha] gene and A1/A2 polymorphism of the CYP17 gene were amplified by polymerase chain reaction, enzyme restriction, and electrophoresis.

Main outcome measure(s) The ER genotypes were classified into A through T (TA repeats, 10-29). The CYP17 genotypes included indigestible (A1 homozygote), heterozygote, and digestible (A2 homozygote). We compared these polymorphism distributions in both groups.

Result(s) The percentage of genotypes D-G (TA, 13-16) in both groups were 10.5%, 29.4%, 13.0%, and 11.3% in group 1 and 7.9%, 16.7%, 19.9%, and 17.6% in group 2. The genotype E (14 TA repeats) is associated with a higher risk of endometriosis. Proportions of A1 homozygote/heterozygote/A2 homozygote for CYP17 were 26.1%/46.2%/27.7% for group 1 and 14.8%/44.5%/40.7% for group 2, respectively. The A1 homozygote and allele were associated with a higher susceptibility of endometriosis.

Conclusion(s) ER[alpha]*14 TA repeats and the CYP17* A1 allele are associated with an increased risk of endometriosis. Both polymorphisms are useful markers for predicting endometriosis susceptibility.


http://www.sciencedirect.com/science/article/B6T6K-4FM9D09-1S/2/85ab2a38941778472772a0efdfbf983ea

Epidermal growth factor receptor (EGFR) is a regulator of angiogenesis and mediator of sex steroid-induced cell growth and differentiation. We observed that EGFR gene 2073*T-related genotypes and allele are associated with higher susceptibilities to endometriosis and leiomyoma.

ObjectiveTumor necrosis factor-[alpha] (TNF-[alpha]), a proinflammatory cytokine, plays an important role in the process of autoimmune diseases. p53 is related to the regulation of cell growth and prevention of carcinogenesis. We propose to investigate whether gene polymorphisms for TNF-[alpha]-308 promoter and p53 could be used as markers of susceptibility in leiomyomas.

DesignProspective basic study.

SettingDepartments of gynecology and genetics in a medical center.

Patient(s)Group 1: leiomyoma (n = 159); group 2: non-leiomyoma (n = 131).

Intervention(s)Genomic DNA was obtained from peripheral leukocyte. The TNF-[alpha] and p53 gene polymorphisms were amplified by polymerase chain reaction (PCR), enzyme restriction, and electrophoresis.

Main outcome measure(s)Two gene polymorphisms were identified: [1] the A (cuttable)/G (uncuttable) polymorphisms of the TNF-[alpha] gene on chromosome 6p21.3; [2] A (cuttable)/P (uncuttable) polymorphisms of the p53 gene on chromosome 17p. Genotype and allelic frequencies in both groups were compared.

Result(s)Genotype distribution and allele frequency of TNF-[alpha] gene polymorphism in both groups were significantly different. Proportions of A homozygote/heterozygote/G homozygote for TNF-[alpha] in both groups were: (group 1) 61%/34.6%/4.4% and (group 2) 81.7%/14.5%/3.8%. Proportions of allele A/G for TNF-[alpha] in both groups were: (group 1) 78.3%/21.7% and (group 2) 88.9%/11.1%. Distributions of p53 polymorphisms in both groups were not different. The proportions of A homozygotes/heterozygotes/P homozygotes for p53 were (group 1) 32.7%/42.1%/25.2% and (group 2) 28.2%/48.9%/22.9%. Conclusion(s)G homozygote and G allele for TNF-[alpha] promoter are related to a higher risk of leiomyomas. The p53 codon 72 gene polymorphism is not associated with the susceptibility of leiomyomas.


ObjectiveTo evaluate the association between leiomyomas and estrogen receptor gene polymorphism. DesignProspective study. SettingDepartment of gynecology and genetics. Patient(s)Women with (n = 159) or without leiomyomas (n = 131). Main outcome measure(s)Polymerase chain reaction was used to detect dinucleotide (thymine-adenine [TA]) repeat polymorphisms upstream of the estrogen receptor gene. Genotypes were classified as A through P according to the number of the TA repeats from 12 to 27. Distributions of TA repeat for estrogen receptor in both groups were compared. Result(s)Genotypes A to E were detected in 10.7%, 18.9%, 15.7%, 16.4%, and 4.4%, respectively, of women with leiomyomas and 4.2%, 9.5%, 20.6%, 19.1%, and 10.3% of women without leiomyomas. Women with genotypes A and B (12 or 13 TA repeats) have a higher risk for leiomyomas, and those with genotype E (16 TA repeats) have a lower risk. Conclusion(s)Estrogen receptor gene polymorphism probably contributes to the pathogenesis of leiomyoma and may predict the susceptibility to leiomyoma. The 12 and 13 TA repeats are associated with a higher risk of leiomyoma.


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http://www.sciencedirect.com/science/article/B6T6K-3V5N66Y-T/2/01a768dc73c5e5ff42e5b6849e44ebc5

Objective: To investigate the role of interleukin-8 (IL-8) in peritoneal fluid of patients with endometriosis in the pathogenesis of endometriosis. Design: Peritoneal fluid was collected by laparoscopy. Endometrial and endometriotic stromal cells were obtained from normal endometrium and from chocolate cyst linings of the ovary. Setting: Department of Obstetrics and Gynecology of Tottori University Hospital, Yonago, Japan. Patient(s): Forty women who underwent either laparoscopy or laparoscopic surgery. Main Outcome Measure(s): The peritoneal fluid concentration of IL-8 was analyzed by enzyme-linked immunosorbent assay, and the correlation between the IL-8 concentration and the extent of active endometriosis was investigated. The effect of IL-8 on cell proliferation was examined by tetrazolium bromide and thymidine incorporation. The expression of IL-8 receptor was examined in stromal cells by reverse transcription polymerase chain reaction. Result(s): The level of IL-8 in peritoneal fluid was significantly higher in patients with endometriosis than in patients without endometriosis. A significant correlation was noted with the extent of active endometriosis. Interleukin-8 significantly increased the number of cells and DNA synthesis in the endometrial and endometriotic stromal cells in a dose-dependent manner. Transcripts of IL-8 receptor type A were detected in stromal cells. Conclusion(s): The present study suggests that IL-8 found in the peritoneal fluid of patients with endometriosis contributes to the pathogenesis of endometriosis.


http://www.sciencedirect.com/science/article/B6T6K-4BY331V-V/2/331b2f3f3f19265e63aeb06ad06bb7e0

Objective: Studies have shown that age at natural menopause is heritable. Mutations in the FSH receptor have been identified in women with premature ovarian failure (POF) and the FSH-receptor gene may, therefore, be considered a candidate gene for (early) menopausal age. This study investigates whether there is linkage between genetic markers in the FSH-receptor region and (early) age at menopause using a sib-pair design. Setting: Sister pairs and their first-degree family members from The Netherlands. Patient(s): The inclusion criteria for a family were natural menopause in upper or lower tail of the distribution of menopausal age in at least two sisters. A total of 126 families with at least one sib-pair were included in this study. Six polymorphic markers encompassing the FSH-receptor gene were genotyped. Intervention(s): None. Main outcome measure(s): Single point and multipoint logarithm of the odds (LOD) scores. Result(s): None of the markers showed evidence in favor of linkage with overall age at natural menopause or early age at natural menopause. Conclusion(s): Possibly, age at natural menopause in the more or less normal range is not part of the spectrum of phenotypes determined by mutations in the FSH-receptor gene. Alternatively, our results might be explained by genetic heterogeneity in the left tail of the distribution of menopausal age. This can limit the chance of finding a genetic locus, especially if this factor has a modest contribution to the phenotype.

http://www.sciencedirect.com/science/article/B6T6K-48S3GMC-G/2/59f2a5c5a1f26d4bdf4368371140a96c

ObjectiveTo determine whether genetic variability in the gene encoding microsomal epoxide hydrolase (EPHX) contributes to individual differences in susceptibility to the development of polycystic ovary syndrome (PCOS).DesignRetrospective case-control study.SettingUniversity-based clinic.Patient(s)One hundred twelve white women with PCOS and 115 healthy controls.Intervention(s)None.Main outcome measure(s)The presence of two single nucleotide polymorphisms (SNPs), T->C (Tyr113His) in exon 3 and A->G (His139Arg) in exon 4, in the EPHX gene. Single point analysis was expanded to pair of loci haplotype analysis to examine the estimated haplotype frequencies of the two SNPs, of unknown phase, in the PCOS and control groups. Estimated haplotype frequencies were assessed using the maximum-likelihood method, using an expectation-maximization algorithm.Result(s)Single point allele and genotype distributions in exon 3 and exon 4 of the EPHX gene were not statistically different between the groups. However, according to the haplotype estimation analysis, we observed a significantly elevated frequency of haplotype C-G (His113-Arg139) in the PCOS group versus the control group. The odds ratio for PCOS associated with the low activity haplotype C-G (His113-Arg139) was 2.28 (95% confidence interval 1.1-4.8).Conclusion(s)The use of two intragenic single nucleotide polymorphisms jointly in haplotype analysis of association demonstrated that the genetically determined low activity haplotype C-G (His113-Arg139) was significantly associated with PCOS.


http://www.sciencedirect.com/science/article/B6T6K-3WK3SS6-D/2/7bc0cc447477ca41cd01edca54de2cfc

Objective: Antiphospholipid antibodies (APA) and other coagulation abnormalities have been associated with an increased risk of venous, arterial, and placental thrombosis and recurrent pregnancy loss (RPL). Factor V Leiden (a point mutation [1691G->A] in the factor V gene), the prothrombin 20210G->A mutation, and homozygosity for a common polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene (677C->T) have been associated with arterial and venous thrombosis and arterial occlusive disease. We explored an association between these markers of thrombophilic states and RPL. Design: Prospective case-control evaluation. Setting: University-associated private practice. Patient(s): Fifty nonpregnant women with three or more pregnancy losses and 50 healthy, nonpregnant controls. Intervention(s): None. Main Outcome Measure(s): Anticardiolipin and antiphosphatidylserine antibodies were detected in serum by ELISA. Polymerase chain reaction was performed to identify the factor V Leiden (1691G->A) mutation, the thermobile MTHFR (677C->T) mutation, and the prothrombin 20210G->A mutation. Result(s): The following were identified by restriction fragment-linked polymorphism analyses: 1 (2%) factor V Leiden heterozygosity; 1 (2%) prothrombin 20210G->A heterozygosity; and 4 (8%) thermolabile MTHFR homozygosity. None of these mutation frequencies in women with RPL were statistically significantly different from controls. Conclusion(s): These data suggest that factor V Leiden, thermolabile MTHFR (677C->T), and prothrombin 20210G->A are not found at an increased frequency in women with a history of early RPL.

http://www.sciencedirect.com/science/article/B6T6K-4DB52W5-13/2/86d243eaf1e9daceecbf556a5ca1f654

Objective
To compare the mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (KDR and flt-1) in the implantation and nonimplantation sites of the human oviduct with ectopic gestation.

Design
Prospective observational study.

Setting
University-based Obstetrics and Gynecology Department.

Patient(s)
Ten women undergoing laparoscopic salpingectomy for tubal pregnancy.

Intervention(s)
The mucosal layer was isolated from the implantation and nonimplantation sites of the oviduct tissue with ectopic gestation. Semiquantitative reverse transcriptase-polymerase chain reaction was performed.

Main outcome measure(s)
The differences in the mRNA expression of VEGF and its receptors between the implantation and nonimplantation sites of the oviduct tissue.

Result(s)
The mRNA expression of VEGF and its receptors, both KDR and flt-1, was significantly higher in the implantation site of the human oviduct with ectopic gestation compared with the nonimplantation site.

Conclusion(s)
The results suggest that VEGF may be the angiogenic factor responsible for the implantation and placentation of an ectopic pregnancy in the oviduct.


http://www.sciencedirect.com/science/article/B6T6K-4BN4RKT-15/2/c4ac3941524b36f4145d94732fbafa0b

Objective
To examine the localization of vascular endothelial growth factor receptors (VEGF-R) and the changes in VEGF-R messenger ribonucleic acid (mRNA) expression in various regions of the oviduct from fertile women throughout the ovulatory cycle.

Design
Prospective observational study.

Setting
University-based obstetrics and gynecology department.

Patient(s)
Twenty-two women who underwent laparoscopic tubal sterilization or hysterectomy for a benign gynecological condition.

Intervention(s)
The mucosal layer was isolated from the oviduct tissue. Immunohistochemistry and a semiquantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) was performed.

Main outcome measure(s)
Immunohistochemical localization of VEGF-R proteins in oviduct tissue, and the differences of VEGF-R mRNA expression in the various regions of the oviduct and in the various stages of the ovulatory cycle.

Result(s)
Immunohistochemical study localized VEGF-R, both KDR and flt-1, in the oviduct luminal epithelium, smooth muscle cells as well as blood vessels within the oviduct. Messenger RNA expression of KDR, but not flt-1, was significantly higher in the ampullary and infundibular regions than in the isthmus. Messenger RNA expression of flt-1, but not KDR, varied significantly in the oviduct along the course of an ovulatory cycle, with the highest level in the periovulatory stage.

Conclusion(s)
These results suggest that the two VEGF receptors may have different roles in the oviduct. Our data support a role for KDR in oviduct angiogenesis whereas flt-1 appears to be important in the temporal regulation of oviductal secretion.

ObjectiveTo investigate the expression of full-length and truncated hCG/LH-receptor mRNA in human endometrium and decidua.

DesignIn vitro experiment.

SettingTertiary university center.

Patient(s)Premenopausal women undergoing hysterectomy because of benign diseases or induced abortions.

Intervention(s)Isolation of RNA from endometrial samples, reverse transcription, selective preamplification of full-length hCG/LH receptor mRNA and several shorter fragments of the receptor gene (exons 1-11, 1-10, and 1-5), nested polymerase chain reaction with internal primers.

Main outcome measure(s) Appropriately sized cDNA product confirmed by sequencing.

Result(s)All samples derived from the proliferative as well as from the early and mid-luteal phases were positive for all four amplification products, suggesting the expression of a full-length hCG/LH receptor mRNA. Only 5 of 8 samples derived from the late secretory phase and 2 of 12 samples derived from early decidua amplified the entire receptor sequence. In contrast, the shortest fragment (exons 1-5), coding for part of the extracellular receptor domain, was amplified in all samples.

Conclusion(s) The data suggest cycle-dependent regulation of hCG/LH-receptor mRNA by changes in the alternative splicing pattern and down-regulation of full-length hCG/LH receptor mRNA in early decidua. The major splicing site appears to be located between introns 5 and 9. Alternative splicing may be a mechanism regulating hCG/LH-receptor down-regulation.


ObjectiveTo determine the role of infections in miscarriages. Chorionic villi from aborted material were subjected to cytogenetic evaluation and analyzed for the presence of Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, human cytomegalovirus (HCMV), adeno-associated virus (AAV), and human papillomaviruses (HPV).

DesignRetrospective study.

SettingUniversity hospital and academic research institution.

Main outcome measure(s) Karyotyping and detection of bacterial and viral DNA by means of polymerase chain reaction (PCR) in placenta specimens.

Result(s) In 54 (50%) of 108 samples the karyotype was normal, in 38 (35%) samples it was abnormal, and in 16 (15%) samples karyotype was undetermined. No U. urealyticum, M. hominis, HCMV, or AAV-2 DNA was detected, while C. trachomatis DNA was detected in one (1%) and HPV DNA in eight (7%) samples. No significant correlation of HPV-positive findings with karyotype status was established.

Conclusion(s) Our findings do not support a role of C. trachomatis, U. urealyticum, M. hominis, HCMV, or AAV infections in miscarriages during the first trimester of pregnancy. However, further investigation should be made to determine a possible involvement of HPVs in the development of genetic abnormalities of the fetus and in miscarriages.

lesions were obtained from patients during laparoscopic surgery. Main outcome measure(s): Clonality analysis used the laser microdissection technique, a phosphoglycerate kinase (PGK) gene polymorphism assay, and an androgen receptor (AR) gene polymorphism assay after digestion of the DNA with methylation-sensitive endonuclease. Result(s): Each ectopic gland of the peritoneal endometriotic lesion showed a monoclonal pattern in both the PGK gene and AR gene assays, but the methylation pattern of the PGK gene and/or AR gene was divergent among adjacent glands in the lesion. These data indicate that the peritoneal endometriotic lesions are multicellular in origin, although individual glands of the lesion are derived from single precursor cells. Conclusion(s): The colored peritoneal endometriotic lesion in the present study was multicellular in origin. Peritoneal endometriotic lesions may thus be initiated by transplantation of a cluster of eutopic endometrial tissues into the pelvis.


http://www.sciencedirect.com/science/article/B6T6K-3VW837TP/2/0f0e01ea6ff5e83c0da725b1f529472

Objective: To analyze the effects of certain herbs on sperm DNA and on the fertilization process. Design: Prospective comparative study. Setting: Clinical and academic research environment. Patient(s): Donor sperm specimens. Intervention(s): Zona-free hamster oocytes were incubated for 1 hour in saw palmetto (Serenoa repens), echinacea purpura, ginkgo biloba, St. John's wort (Hypericum perforatum), or control medium before sperm-oocyte interaction. The DNA of herb-treated sperm was analyzed with denaturing gradient gel electrophoresis. Main Outcome Measure(s): Oocyte penetration and integrity of the sperm BRCA1 exon 11 gene. Result(s): Pretreatment of oocytes with 0.6 mg/mL of St. John's wort resulted in zero penetration. A lower concentration (0.06 mg/mL) had no effect. High concentrations of echinacea and ginkgo also resulted in reduced oocyte penetration. Exposure of sperm to echinacea purpura and St. John's wort resulted in DNA denaturation. In contrast, saw palmetto and ginkgo had no effect. Sperm exposed to 0.6 mg/mL of St. John's wort showed mutation of the BRCA1 exon 11 gene. Conclusion(s): High concentrations of St. John's wort, echinacea, and ginkgo had adverse effects on oocytes. Saw palmetto had no effect. The data suggested that St. John's wort, ginkgo, and echinacea at high concentrations damage reproductive cells. St. John's wort was mutagenic to sperm cells.


http://www.sciencedirect.com/science/article/B6T6K-41GNKR4-14/2/f0eb3a4d4a273ac927da0f3cbd615ef3

Objective: To determine whether meiotic and postmeiotic germ cell gene products could be detected in biopsy specimen from patients with Sertoli cell only (SCO) and maturation arrest. Design: Prospective clinical study. Setting: University-based departments and laboratories. Patient(s): Nine patients, seven with nonobstructive azoospermia (12 biopsies) and two with obstructive azoospermia (controls) (2 biopsies). Intervention(s): Specimens were divided into three parts: IVF laboratory, histology, and molecular analysis. Germ cell-specific messenger RNAs (mRNAs) were detected by extracting total RNA for Northern blotting or reverse transcription-polymerase chain reaction. Main Outcome Measure(s): Detection of meiotic (lactate dehydrogenase C4) and postmeiotic (transition protein 1 and protamine 1 and 2) gene expression
and correlation with histologic and IVF laboratory findings. Result(s): The IVF laboratory identified spermatozoa in 3 of 14 biopsies (controls and severe hypospermatogenesis). Histologically, 6 of 14 biopsies (43%) were diagnosed as SCO, 4 (29%) maturation arrest, 2 (14%) severe hypospermatogenesis, and 2 normal. Molecular analysis showed mRNA for meiotic and postmeiotic genes in 12 of 14 biopsies (86%) (P = .006), of which 4 (67%) in SCO and 3 (75%) in maturation arrest. Conclusion(s): Differentiated germ cells are present in biopsies of men histologically diagnosed as SCO. Absence of these molecular markers strengthens the histologic diagnosis and helps the physician in counseling the infertile couple.


http://www.sciencedirect.com/science/article/B6T6K-44C0HF9-X/2/52db759e0db98a44f2d6731fa11d4fab

Objective: To investigate a possible association between the carrier frequency of the N314D mutation in the galactose-1-phosphate uridyl transferase (GALT) gene and endometriosis and linkage to the short arm of chromosome 9, where the GALT gene resides. Design: Association and linkage study. Setting: Population material collected for case and family studies in endometriosis. Patient(s): Women diagnosed with endometriosis by laparotomy or laparoscopy. Intervention(s): Association with the GALT gene investigated by genotyping 85 affected women and 213 unrelated control women and a scan for linkage to chromosome 9 in 205 women from 64 families with endometriosis. Main Outcome Measure(s): Multipoint parametric lod scores and frequency of alleles. Result(s): There was no significant difference in allele frequency for the N314D polymorphism in patients compared with control subjects. No evidence for linkage was found to chromosome 9p, where the GALT gene resides. Conclusion(s): The experiments reported herein provide no evidence supporting involvement of the GALT locus in the development of endometriosis.


http://www.sciencedirect.com/science/article/B6T6K-3XRP7P8-J/2/050c4fc7b3e874d105a1de7b18576bd9

Objective: To explore endometriosis-related molecules in patients with use of differential display analysis. Design: Prospective study. Setting: Nagoya City University Medical School, Nagoya, Japan. Patient(s): Women with endometriosis (n = 27) and without endometriosis (n = 21). Intervention(s): Surgery was scheduled in the proliferative or secretory phase of the menstrual cycle. Main Outcome Measure(s): Differentially expressed products of endometrioma samples were sequenced at nucleotides. One of the candidate genes, secretory leukocyte protease inhibitor (SLPI) gene, was analyzed with use of in situ hybridization and Northern blot analyses. Distribution of SLPI was determined by immunohistochemistry, and the amount of SLPI in the peritoneal fluid and serum was measured by ELISA. Result(s): Distinct expression of SLPI messenger RNA could be detected in the endometrial-type epithelium of extratube endometriotic tissues and in the eutopic endometrium of women with endometriosis. SLPI was localized in the endometrial-type epithelium of endometriomas immunohistochemically. The amount of SLPI in the peritoneal fluid was markedly elevated in the endometriosis group (91.6 +/- 6.6 ng/mL compared with 68.4 +/- 5.3 ng/mL in the controls). Conclusion(s): Secretory leukocyte protease inhibitor may be involved in the pathogenesis of endometriosis.

http://www.sciencedirect.com/science/article/B6T6K-3V5N66Y-N/2/ee957afe01a6583be59c6ebd31a40c71

Objective: To examine the frequency of anomalies of the vas deferens and the frequency of mutations of the cystic fibrosis transmembrane regulator (CFTR) gene in male candidates for intracytoplasmic sperm injection (ICSI) who had severe oligoasthenoteratozoospermia.

Design: The clinical data for male candidates for ICSI were studied. The three most frequent cystic fibrosis (CF)-causing CFTR mutations in the Dutch population ([:Delta:]F508, A455E, and G542X) and the three most frequent CFTR mutations potentially causing congenital bilateral absence of the vas deferens (CBAVD) in the Dutch population ([:Delta:]F508, R117H, and IVS8-5T) were analyzed. Delta I507 is also detected by the [:Delta:]F508 test. Samples of DNA from patients identified as CFTR mutation carriers were subjected to denaturing gradient gel electrophoresis analysis with use of a two-dimensional electrophoretic technique.

Setting: University-based center for reproductive medicine and clinical genetics.

Patient(s): Male candidates for ICSI who had oligoasthenoteratozoospermia and no history of operative sterilization and refertilization. Males with a chromosomal aberration or a Y-chromosome microdeletion were excluded.

Intervention(s): Semen and blood samples were collected from the patients at their first visit to the clinic.

Main Outcome Measure(s): Frequency of anomalies of the vas deferens and frequency of mutations of the CFTR gene in male candidates for ICSI who had oligoasthenoteratozoospermia.

Result(s): None of the patients had abnormalities of the vas deferens at physical examination. In 4 of the 150 chromosomes (75 patients), a CFTR mutation was found, yielding a CFTR mutation frequency of 2.7% (95% confidence interval, 1.0-6.7%). None of the patients had two CFTR mutations.

Conclusion(s): The frequency of congenital abnormalities of the vas deferens in patients with oligoasthenoteratozoospermia is low. The frequencies of the CFTR mutations identified in this cohort did not differ significantly from the frequencies found in the normal Dutch population.


http://www.sciencedirect.com/science/article/B6T6K-496N8HW-7/2/83f88bf3c112652fb55f0dd791eb10e6

ObjectiveTo gain a comprehensive view of the gene expression and regulation involved in uterine leiomyomata and matched normal myometrium using oligonucleotide microarray-based hybridization analysis.

Design: Retrospective analyses of tissue obtained in a prospective randomized clinical study.

Setting: Academic institution.

Patient(s): Seven patients with leiomyomata scheduled for surgery during the proliferative phase.

Intervention(s): Seven paired samples of leiomyomata and adjacent myometrium were obtained from patients undergoing hysterectomy.

Main outcome measure(s): The total RNA extracted from leiomyomata and myometrium was used for gene expression profiling of 6,800 human genes using high-density oligonucleotide microarrays. In addition, reverse transcriptase-semiquantitative polymerase chain reaction and immunohistochemistry were used to validate tumor-specific gene expression.

Result(s): A comparison of expression patterns in each paired sample revealed 68 genes significantly up- or down-regulated in each paired tissue sample, of which 23 genes showed increased expression and 45 showed decreased expression in leiomyomata compared with normal myometrium. Cluster analysis supported the relevance of these candidate genes for distinguishing between normal myometrium and leiomyomata biologic
activity. Conclusion(s) Expression profiling of uterine leiomyomata using high-density oligonucleotide microarrays yields signature patterns that reflect the distinctive differences between normal human myometrium and leiomyomata during the proliferative phase. These observations suggest that a number of genes are involved in the tumorigenesis of leiomyomata.


http://www.sciencedirect.com/science/article/B6T6K-47T5CDW-18/2/aa5c336ef74528cd5e6a7bd4726a46a8

Objective To screen for mutations in the GnRH receptor gene in a case of complete hypogonadotropic hypogonadism (HH) with GnRH resistance. Design Case report. Setting A university hospital. Patient(s) A male patient with the complete form of HH without anosmia. Intervention(s) Physical examination and laboratory and genetic studies. Main outcome measure(s) Gonadotropins at the basal state and after GnRH administration and GnRH receptor DNA sequencing. Result(s) A novel missense mutation, localized in the first amino acid of the extracellular loop found in the heterozygous state, and another mutation, Arg139His (R139H), located in the conserved aspartate-arginine-serine motif at the junction of the third transmembrane and second intracellular loop of the GnRH receptor, were identified in the homozygous state. Pedigree studies reveal that both parents were heterozygous for R139H, while the mother carried the missense mutation at codon 1(M1T). Conclusion(s) GnRH receptor mutations may account for a larger proportion of cases of HH than previously thought. The phenotypic spectrum of HH seems to vary, and this heterogeneity may be related, at least in part, to the degree of impaired biological activity of the mutated GnRH receptor caused by the allelic type of mutations.


Objective To determine the clonal origins of endometriotic lesions using laser capture microdissection and PCR-based HUMARA assay. Design Molecular genetic study of human tissue. Setting Molecular genetics laboratory in an academic setting. Patient(s) Twenty patients with endometriosis. Forty specimens of endometriotic lesions from these patients and one specimen of normal endometrium were analyzed. Intervention(s) Laser capture microdissection was used to harvest epithelial cells from single and multifocal endometrial lesions from paraffin-embedded and frozen tissues, and their clonality was determined with the HUMARA assay. Main outcome measure(s) Polymerase chain reaction-based HUMARA assay of clonality. Result(s) Thirty-eight specimens were polymorphic and thus informative. Most specimens were monoclonal, as determined by the HUMARA assay. In four specimens of multifocal lesions, polyclonality was detected, but upon more refined microdissections and further analyses, we found that each focus was monoclonal individually. Conclusion(s) Previously reported polyclonality is very likely to be attributed to the pooling of multifocal lesions or contamination of normal tissues. These results suggest that endometriotic lesions were monoclonal in origin, and in the case of multifocal lesions, each focus originates monoclonally; hence, different foci have independent origins. The monoclonality of endometriotic lesions suggests that they may carry neoplastic potentials, and the apparent independent origins of multifocal lesions suggest that reconstruction of individual lesion
histories may help us to understand the initiation and progression of endometriosis.


http://www.sciencedirect.com/science/article/B6T6K-4DH19R3-V/2/42477cd1302ccf29e0bd58c50d1caee64

ObjectiveTo test early-gestation human placenta, a human trophoblast cell line, mouse eggs, preimplantation embryos, and a mouse trophoblast cell line for the expression of mRNA transcripts for stress-activated protein kinase/c-Jun N-terminal kinase (SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and SAPK[beta]/JNK3).DesignWhole RNA was isolated from the tissue sources listed above and control tissues, and reverse transcription-polymerase chain reaction (RT-PCR) was performed to assay for the qualitative and semiquantitative presence of SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and SAPK[beta]/JNK3.SettingNone.Patient(s)None.Intervention(s)None.Main outcome measure(s)The presence and magnitude of amplimer amounts in gels or gene hybridization on Affymetrix cDNA arrays of RT-PCR products of reactions for SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and SAPK[beta]/JNK3.Result(s)SAPK[gamma]/JNK1 and SAPK[alpha]/JNK2 mRNA transcripts are present in early-gestation human placenta, a human trophoblast cell line, mouse eggs, preimplantation embryos, and a mouse trophoblast cell line at levels similar to positive control levels. SAPK[alpha]/JNK2 is expressed at the highest level of the three transcripts in the family. SAPK[beta]/JNK3 is present at levels that are 1/100-1/1,000 those of the positive control and in some cases at the apparent level of the negative control (previously measured by the less-sensitive Northern blot analysis). Analysis with an Affymetrix cDNA array suggested that SAPK[alpha]/JNK2 and 38 kDa mitogen-activated protein kinase had the highest mRNA expression measured for each of three family members.Conclusion(s)Mitotic placental trophoblast cell lines and primary conceptus/embryo samples containing early placental trophoblasts express SAPK[alpha]/JNK2 at higher levels than SAPK[gamma]/JNK1, but not (only low background levels of) SAPK[beta]/JNK3 mRNA transcripts. This suggests that SAPK[gamma]/JNK1 and SAPK[alpha]/JNK2 may be important mediators of stress-induced responses in early implanting conceptuses that could mediate embryo loss.

Fish & Shellfish Immunology (3)


http://www.sciencedirect.com/science/article/B6WFN-48B5JPD-8/2/d1cf80edeb3a4f103d11c34607def1c6e

In this study we report the differences in distribution and retention of Aeromonas salmonicida antigens after vaccination with two different vaccines. Parr of Atlantic salmon (Salmo salar) were given intraperitoneal injections of either a commercial, monovalent furunculosis vaccine (Apoject) or live, attenuated A. salmonicida ([Delta]aroA). Fish were sampled at weeks 2, 4 and 12 post-
vaccination and head kidney and spleen were collected. Presence of LPS and 16S rDNA in isolated leukocytes were investigated by immunocytochemistry and polymerase chain reaction (PCR). 16S rDNA was detected in head kidney and spleen of all [Delta]aroA vaccinated and most Apoject-vaccinated fish at weeks 2 and 4. At week 12, 16S rDNA was detected in none of the [Delta]aroA vaccinated fish, but it was detected in head kidney of 75% of Apoject-vaccinated fish. LPS was detected in both vaccination groups at all sampling times, but most frequently in the [Delta]aroA vaccinated fish (in head kidney 75-83% vs. 50%, in spleen 58-67% vs. 17-25%).


http://www.sciencedirect.com/science/article/B6WFN-48PDMG3-4/2/371bba9af1e9ee06a718311fe1a885e6

A cDNA containing the gene for Japanese flounder IgD consisted of 3240 bp encoding 998 amino acid residues. The amino acid sequence of the constant region of Japanese flounder IgD shares 38-80% identity with the sequences of previously reported teleost IgDs. The structure of the constant region of Japanese flounder IgD, which contains the [mu]1, [delta]1, [delta]2, [delta]3, [delta]4, [delta]5, [delta]6, [delta]7, and TM regions, is similar to the structures of the constant regions of the IgDs of channel catfish and Atlantic salmon. Southern blot hybridisation showed that the Japanese flounder IgD gene exists as a single locus. The Japanese flounder IgD gene was mainly detected in peripheral blood leucocytes (PBLs) and small amounts were detected in the spleen, head and trunk kidney, although IgM mRNA was detected in similar amounts in PBLs, the head kidney, and spleen. The copy number of IgM mRNA in Japanese flounder PBL was 56-fold higher than that of IgD.


Naked circular plasmid DNA containing the cytomegalovirus (CMV)-promoter-driven lacZ reporter gene (pCMV-LacZ) was injected in the epaxial muscle of gilthead sea bream (Sparus aurata). A mosaic pattern of expression of [beta]-galactosidase ([beta]-gal) in the myofibres at the site of injection was visualised by in situ histochemical staining using 5-bromo-4-chloro-3-indolyl-[beta]--galactopyranoside. As measured by o-nitrophenyl-[beta]--galactopyranoside assay, [beta]-gal enzymatic activity was found to steadily increase for at least 50 days post injection (p.i.) in pCMV-LacZ-injected muscle. In parallel, foreign DNA was detected by polymerase chain reaction in injected muscles (but not in other tissues) up to 60 days p.i., persisting most probably in an extrachromosomal, non-replicative, circular form. Neither [beta]-gal activity nor pCMV-LacZ-related amplification products were found 90 days p.i. Antibodies against [beta]-gal were demonstrated in pCMV-LacZ-injected fish sampled 45 days p.i. The results suggest that intramuscular delivery of foreign genes represents a realistic approach for DNA vaccine technology for the prevention of infectious diseases in gilthead sea bream.
In food poisoning, detection of the nature of causative agent is important for management of trauma and forensic investigation. Most of the methods in clinical toxicology are developed for detection of toxins and poisons. A RAPD-based method has been described for detection of species of animal from its morphologically unrecognizable fragments, recovered from food substances, consumption of which caused even death. Pre-mixed RAPD reaction beads and six RAPD primers were used in PCR analysis. Among six RAPD primers used, any one of them was sufficient in resolving this practical forensic situation. But to enhance the probability values for matching in the present study of fixing identity of an animal, six set of market available RAPD primers were used. This is the first report of a forensic application of RAPD DNA typing in identification of charred skeleton remnants of Lizard sp. in food material. Furthermore unique amplicons were generated for different reptilian species, which can be used as species specific markers for species identification in forensic situation, however no variations among individuals of same species were observed.

Food Control  (6)


Regulations for the use and labeling of genetically modified organism products and derived ingredients are being implemented worldwide, what demands reliable and accurate methods to detect genetically modified organisms (GMO) in raw materials and food products. This study aimed at monitoring products derived from GMO in the Brazilian market using detection methods
for the presence of Roundup Ready soybean, Bt176 and MON 810 maize. The results demonstrate for the first time the presence of GM-soy in Brazilian food products, reinforcing the need for the development of accurate quantitative methods in routine analyses.


http://www.sciencedirect.com/science/article/B6T6S-4CYGS6G-5/2/d7ba90f893ca06af15571240305c8111

Work reported here shows the outcome of improvements made to a published PCR-RFLP approach for fish species identification. The objective of the improved method was to replace the gel-electrophoretic steps for fragment separation, detection and analysis, by employing a chip-based capillary electrophoresis (CE) system. Fragment resolution on the system was sensitive, with detection of small fragments not observed with the published conventional gel-based method. Experimental repeatability was less than 3%, allowing species identification without the need to run reference materials with every sample. Using DNA admixtures, the discrimination of 5% salmon DNA in trout DNA was readily achieved. Results showed that the CE system was quick and easy to use, providing post-restriction digestion results for 12 samples within 40 min. This, along with the relatively low cost of the instrument, should make this method suitable for use in a wide range of analytical laboratories involved with species identification.


http://www.sciencedirect.com/science/article/B6T6S-49H1M6C-2/2/187305c10f6039673a3f028749aa600f

The genetically modified (GM) Roundup Ready(R) soybean event GTS 40-3-2 contains the bacterial gene 5-enol-pyruvylshikimate-3-phosphate synthase. A 534 bp rearrangement of the DNA in the 3' region flanking the functional insert likely occurred as a consequence of the insertion event. The structure of the DNA surrounding the insertion has not been fully characterized. A semi-nested PCR method identified the rearranged soybean DNA in samples of raw, partially processed and highly processed food at a level of 0.01%. The 534 bp rearranged segment contained a contiguous portion at least 238 bp long that was also identified in non-GM soybeans. Specific combinations of semi-nested PCR primers differentiated GM and non-GM soybean DNA. These studies confirm that the rearranged DNA originates from the soybean genome and does not involve the introduction of non-soybean genetic material.


http://www.sciencedirect.com/science/article/B6T6S-3Y2F99V-9/2/2c585067a6abe9ad8619ede46b04143f

Two different PCR-based approaches for the quantitative analysis of genetically modified organism (GMO) - components in foods are presented using Soybean derived samples as an example. The first method - a double competitive PCR - is well suited to determine threshold levels of GMO content in food. The other - PCR on-line measurement - is suited to determine
ratios of transgenic versus non-transgenic component. Both methods provide a means to alleviate the problems of standardisation encountered with simple qualitative PCR approaches and will allow to cope with threshold levels for GMO, once issued by legislative bodies.

**Food Microbiology**  (2)


http://www.sciencedirect.com/science/article/B6WFP-4D3W9F4-P/2/37e9778ec2e71dd1ce8c100ff726fb70

Listeria monocytogenes is a foodborne pathogen frequently present in ripened soft cheeses. Forty-three strains of L. monocytogenes isolated from the rind of ripened Gorgonzola cheeses produced in 24 different dairy plants were characterized by biotyping, serotyping, and molecular typing. Biotyping was performed by studying two phenotypes closely associated with virulence, such as hemolytic and phospholipase C activities. Traditional typing techniques did not allow a discrimination among the 43 strains studied. All strains showed a good hemolytic activity on blood agar, and only slight differences were observed when titration of hemolytic activity of culture supernatants was performed. Also phospholipase activities were quite similar for all the strains. Concerning serotyping, all strains belonged to serotype 1/2a. The molecular characterization was performed by RAPD-PCR. Combined cluster analysis following PCR amplification experiments allowed to group L. monocytogenes strains into few distinguishable profiles. At a level of similarity of 80%, the 43 strains were grouped into only 5 composite profile groups. Although isolated in 24 different plants, the presence of a few closely related strains demonstrated a possible relationship between these cheese isolates; a special ability of these strains to adapt to Gorgonzola cheese processing environment could be suggested.


http://www.sciencedirect.com/science/article/B6WFP-49XWF1-C/2/9fbd0bc0819cd0b74e434cc9cd98c0b7

E. coli O157 is a foodborne pathogen responsible for serious human illnesses, such as hemorrhagic colitic and hemolytic uremic syndrome. Ground beef products are among the foods that are more commonly contaminated, and the strains isolated have been frequently found to carry virulence factors of this pathotype. This paper reports the results of serotyping assays and of investigations performed to screen for virulence factors of 10 E. coli O157 strains isolated from fresh sausages purchased at retail meat outlets in various provinces of Apulia (southern Italy). The presence of verocytotoxins was assessed on VERO cells and ELISA tests. Multiplex PCR assays were performed for the eae, stx1, stx2 and hlyA genes. Six of the 10 strains examined presented the H7 antigen and all of them proved to be potentially pathogenic due to the presence of individual or multiple virulence factors.

http://www.sciencedirect.com/science/article/B6T6V-3RYCKHY-6/2/40d09d1b973e0141e32e949044cc9b2

The starter used in the production of Parmesan cheese consists of mixed-strain whey cultures of thermophilic rod lactic acid bacteria, where several strains of each species can be found. The present work was undertaken to demonstrate the effects on the strain composition of interactions among individual lactobacilli grown together in cheese whey. Three strains belonging to Lactobacillus helveticus and Lactobacillus delbrueckii subsp. bulgaricus and subsp. lactis were cultured in Parmesan cheese whey and counted by a molecular approach based on Random Amplified Polymorphic DNA (RAPD) fingerprinting. Results showed interactions among the three lactobacilli as confirmed by comparison of growth kinetics and pH behaviour of the respective strains in mixed-strain culture with single-strain cultures. The present work underlines the importance of noting strain-to-strain interactions among thermophilic lactobacilli in whey co-cultures.


http://www.sciencedirect.com/science/article/B6T38-3YNXYM6-7/2/f2c1155892407643f6614809edd9f5bb

We tested the long-term effects of sublethal oxidative stresses on replicative senescence. WI-38 human diploid fibroblasts (HDFs) at early cumulative population doublings (CPDs) were exposed to five stresses with 30 [mM] tert-butylhydroperoxide (t-BHP). After at least 2 d of recovery, the cells developed biomarkers of replicative senescence: loss of replicative potential, increase in senescence-associated [beta]-galactosidase activity, overexpression of p21Waf-1/SDI-1/Cip1, and inability to hyperphosphorylate pRb. The level of mRNAs overexpressed in senescent WI-38 or IMR-90 HDFs increased after five stresses with 30 [mM] t-BHP or a single stress under 450 [mM] H2O2. These corresponding genes include fibronectin, osteonectin, [alpha]1(I)-procollagen, apolipoprotein J, SM22, SS9, and GTP-[alpha] binding protein. The common 4977 bp mitochondrial DNA deletion was detected in WI-38 HDFs at late CPDs and at early CPDs after t-BHP stresses. In conclusion, sublethal oxidative stresses lead HDFs to a state close to replicative senescence.

The detrimental role of oxidative stress has been widely described in tissue damage caused by ischemia-reperfusion. A nonenzymatic, reactive oxygen species-related pathway has been suggested to produce 8-iso-prostaglandin F2[alpha] (8-iso-PGF2[alpha]), an epimer of prostaglandin F2[alpha] (PGF2[alpha]), which has been proposed as an indicator of oxidative stress. Using an in vivo ischemia-reperfusion model in rat kidneys, we investigated intrarenal accumulation of 8-iso-PGF2[alpha] and PGF2[alpha]. Both prostanoids accumulated in the ischemic kidney and disappeared upon reperfusion. In addition, a nonselective (acetylsalicylic acid) or selective cyclooxygenase (COX) 1 inhibitor (SC-560) completely abrogated the 8-iso-PGF2[alpha] and PGF2[alpha] formation in kidneys subjected to ischemia. COX2 inhibition had no effect on the production of these prostanoids. Therefore the two metabolites of arachidonic acid seemed to be produced via an enzymatic COX1-dependent pathway. Neither COX overexpression nor COX activation was detected. We also investigated renal glutathione, which is considered to be the major thiol-disulfide redox buffer of the tissue. Total and oxidized glutathione was decreased during the ischemic period, whereas no further decrease was seen for up to 60 min of reperfusion. These data demonstrate that a dramatic decrease in antioxidant defense was initiated during warm renal ischemia, whereas the 8-iso-PGF2[alpha] was related only to arachidonate conversion by COX1.


Deletions of mitochondrial DNA (mtDNA) are associated with aging and several chronic diseases. We have reported heterogeneous mutations between base pair 8468 and 13446 in mtDNA, the region known as the "common" deletion, in muscle of older humans with impaired glucose tolerance or diabetes mellitus. To further characterize potential effects of age and glycemia on mtDNA integrity, we studied corpulent JCR:LA-cp rats that are characterized by insulin resistance, hyperinsulinemia, and hyperlipidemia, factors strongly associated with both aging and cardiovascular disease. In addition to skeletal muscle, we isolated vascular smooth muscle cells (VSMC) from aortas of 6-, 12-, and 17-month-old rats and exposed them to 5-, 25-, 62-, and 100-mM glucose or a combination of hypoxanthine (100 [mu]M) and xanthine oxidase (0.025 U/ml) to generate reactive oxygen species in separate cultures. Long- and short-fragment and nested polymerase chain reaction was used to detect mutations in the common deletion region. The data demonstrate that aging and the cp genotype confer susceptibility to mtDNA deletions in vivo and that high glucose concentrations can induce mtDNA mutations in vitro. Accordingly, aging and glucose-related oxidative stress and possibly hyperinsulinemia may contribute to alterations in mitochondrial gene integrity and the cp genotype appears to increase the susceptibility of muscle to the age-related accumulation of mtDNA mutations.

Ascorbic acid (AA) metabolism in streptozotocin (STZ)-induced diabetic rats was determined by examining urinary excretion, renal reabsorption, reductive regeneration, and biosynthesis of AA at 3 and 14 days after STZ administration. AA concentrations in the plasma, liver, and kidney of the diabetic rats were significantly lower than those of controls on d 3, and decreased further as the diabetic state continued. Hepatic AA regeneration significantly decreased in the diabetic rats on d 3 in spite of increased gene expressions of AA regenerating enzymes and was further reduced on d 14. Hepatic activity of L-gulono-gamma-lactone oxidase, a terminal enzyme of hepatic AA biosynthesis, also decreased significantly on d 3 and decreased further on d 14. Urinary excretion of AA was significantly increased on d 3, with an increase in urine volume but no change in gene expressions of renal AA transporters (SVCT1 and SVCT2). Urinary excretion of AA was normalized on d 14. The results suggest that impaired hepatic and renal regeneration, as well as increased urinary excretion and impaired hepatic biosynthesis of AA, contributed to the decrease in AA in plasma and tissues of STZ-induced diabetic rats.


http://www.sciencedirect.com/science/article/B6T38-4B3JMTS-1/2/daa42d85f1c962b5021bb1c0ab112f57

Ionizing radiation-induced adverse biological effects impose serious challenges to astronauts during extended space travel. Of particular concern is the radiation from highly energetic, heavy, charged particles known as HZE particles. The objective of the present study was to characterize HZE particle radiation-induced adverse biological effects and evaluate the effect of -selenomethionine (SeM) on the HZE particle radiation-induced adverse biological effects. The results showed that HZE particle radiation can increase oxidative stress, cytotoxicity, and cell transformation in vitro, and decrease the total antioxidant status in irradiated Sprague-Dawley rats. These adverse biological effects were all preventable by treatment with SeM, suggesting that SeM is potentially useful as a countermeasure against space radiation-induced adverse effects. Treatment with SeM was shown to enhance ATR and CHK2 gene expression in cultured human thyroid epithelial cells. As ionizing radiation is known to result in DNA damage and both ATR and CHK2 gene products are involved in DNA damage, it is possible that SeM may prevent HZE particle radiation-induced adverse biological effects by enhancing the DNA repair machinery in irradiated cells.


http://www.sciencedirect.com/science/article/B6T38-3Y6PRWS-20/2/fceb01988db94e5372e99fa8af6deb20

Because reactive oxygen species have been implicated in the pathogenesis of various hyperproliferative and inflammatory diseases, the mRNA expression of the antioxidant enzyme superoxide dismutase was studied in psoriatic skin tissue. By using reverse transcription-PCR we found similar expression of copper, zinc superoxide dismutase (CuZnSOD) in the involved vs. uninvolved psoriatic skin. In contrast, the level of the manganese superoxide dismutase (MnSOD) mRNA message was consistently higher in lesional psoriatic skin as compared to adjacent uninvolved skin and healthy control skin. Parallel investigation of those cytokines that are thought to be direct or indirect inducers of the MnSOD activity revealed an increased mRNA expression of IL-1[beta], TNF-[alpha], and GM-CSF in lesional psoriatic skin. To study if these cytokines exert a direct effect on dismutase expression in epidermal cells, human keratinocytes in culture were
challenged with IL-1[beta], TNF-[alpha], and GM-CSF. It was found that IL-1[beta] and TNF-[alpha], but not GM-CSF, induced the mRNA expression of MnSOD, and an additive effect was demonstrated for the two former cytokines. Further, the expression of both CuZnSOD and MnSOD transcripts was similar in cultured keratinocytes maintained at low differentiation (low Ca2+ medium) and cells forced to terminal differentiation (by high Ca2+ medium). Our results indicate that the abnormal expression of MnSOD mRNA in lesional psoriatic skin is not directly linked to the pathologic state of keratinocyte differentiation in the skin. It seems more likely that the cutaneous overexpression of MnSOD in psoriatic epidermis represents a protective cellular response evoked by cytokines released from inflammatory cells invading the diseased skin.


http://www.sciencedirect.com/science/article/B6T38-4668X49-G/2/8af966e6ad94f360d0b8bd58b9f249a5

Chronic ethanol consumption is associated with increased protein oxidation and decreased proteolysis in the liver. We tested the hypothesis that even single-dose treatment with ethanol or bromotrichloromethane causes increased protein oxidation and a distinct proteolytic response in cultured hepatocytes. HepG2 cells were treated for 30 min with ethanol, H2O2 and bromotrichloromethane at various nontoxic concentrations. Protein degradation was measured in living cells using [35S]-methionine labeling. Protein oxidation, and 20S proteasome activity were measured in cell lysates. Oxidized proteins increased immediately after ethanol, H2O2, and bromotrichloromethane exposure, but a further significant increase 24-h after exposure was observed only following ethanol and bromotrichloromethane treatment. All three reagents caused a significant increase of the overall intracellular proteolysis at rather low concentrations, which could be suppressed by the proteasome inhibitor lactacystin. A decline of proteolysis observed at higher—subtoxic—concentrations was not related to decreased proteasome activity. Preincubation with ketoconazole or 4-methylpyrazole completely prevented the ethanol- and bromotrichloromethane-induced but not the H2O2-induced protein oxidation and proteolysis, suggesting strongly an enzyme-mediated generation of reactive oxygen species. In conclusion single-dose exposure with ethanol or haloalkanes causes increased protein oxidation followed by an increased proteasome-dependent protein degradation in human liver cells.


http://www.sciencedirect.com/science/article/B6T38-4BS08TP-4/2/f0c5f92276cbcd476ca827326ad52af9

Scavenger receptors recently have been related to Alzheimer's disease, although it is still unclear whether they contribute to the pathogenesis of the disease or reflect an inflammatory response to the deposition of amyloid [beta]-protein (A[beta]). In this study we demonstrate that CD36, a class B scavenger receptor, is highly expressed in the cerebral cortex of Alzheimer's disease patients and cognitively normal aged subjects with diffuse amyloid plaques compared with age-matched amyloid-free control brains. Moreover, in vitro experiments indicated that A[beta] is able to induce CD36 expression in neuronal cells after 24 h treatment. The interaction between CD36 and A[beta] has been reported to trigger oxidant production by macrophages and microglia. In line with this observation, we found an increased presence of nitrated proteins in brains showing A[beta] loads and CD36 overexpression, independent of the occurrence of Alzheimer's disease pathologic features.
The amounts of superoxide and hydrogen peroxide generated by mitochondria under physiological conditions can be enhanced by cellular stress. This study tested the hypothesis that the response to hemin-induced stress, which includes heme oxygenase-1 (HO-1) induction, predisposes to oxidative damage of mitochondrial DNA (mtDNA). Hepatic mitochondria from control, hemin-, and CO-exposed rats were incubated with tert-butyl hydroperoxide (tert-BH) or the NO donor 1,2,3,4-oxatriazolium, 5-amino-3- (3,4-dichlorophenyl)-chloride (GEA 3162). Mitochondrial total and oxidized glutathione (GSH and GSSG), total and free iron, and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-OHdG) were determined with and without oxidants. As expected, oxidation by tert-BH induced significant GSH depletion and increased amounts of free iron and 8-OHdG. Oxidant exposure rapidly produced a large mtDNA deletion involving the coding regions for cytochrome c oxidase (COX 1) and NADH dehydrogenase (ND1 and ND2). Hemin and CO greatly exacerbated susceptibility to the deletion of mtDNA by tert-BH, and this was attenuated by preincubation with GSH methyl ester. Analysis of mitochondria-associated proteins Bax and Bcl-xl in hemin- and CO-exposed rats showed significant responses, revealing interactions with apoptotic pathways. Thus, hemin-induced mitochondrial events sensitize a specific region of the mitochondrial genome to deletion, which is related to depletion of GSH and is not explained by effects of CO. This mtDNA damage is associated with altered expression of mitochondrial cell death proteins, thereby suggesting a novel mechanism for systemic or environmental pro-oxidants to influence apoptosis.

Vitamin E is the primary lipophilic antioxidant in mammals. Lack of vitamin E may lead to an increase of cytotoxic phospholipid-peroxidation products (PL-Ox). We could previously show that alimentary vitamin E-depletion in rats did not change the concentrations of dienes, hydroperoxides, and platelet-activating factor-related oxidation products in alveolar type II cells (TII cells). We hypothesized that vitamin E deficiency increases the activity of enzymes involved in the degradation of PL-Ox. Degradation of PL-Ox may be catalyzed by phospholipase A2, PAF-acetylhydrolase, or peroxiredoxins (Prx's). Alimentary vitamin E deficiency in rats increased the expression of Prx-1 at the mRNA and protein levels and the formation of Prx-SO3, but it did not change the expression of Prx-6 or the activity of phospholipase A2 and PAF-acetylhydrolase in TII cells. H2O2-induced oxidative stress in isolated TII cells activated protein kinase C[alpha] (PKC[alpha]) and increased the expression of Prx-1 and Prx-6. Inhibition of PKC[alpha] in isolated TII cells by long-time incubation with PMA inhibited PKC[alpha] and Prx-1 but not Prx-6. We concluded that the expression of Prx-1 and -6 is selectively regulated in TII cells; PKC[alpha] regulates the expression of Prx-1 but not Prx-6. Prx-6 expression may be closely linked to lipid peroxidation.
Fungal Genetics and Biology (8)


http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-C/2/9b5a34e99f7f3537ef29dc92daaaf45ba


http://www.sciencedirect.com/science/article/B6WFV-4DW98YY-3/2/265f1d644ab17c87ad9265abaf2be78f

A gene for the Alternaria major allergen, Alt a 1, was amplified from 52 species of Alternaria and related genera, and sequence information was used for phylogenetic study. Alt a 1 gene sequences evolved 3.8 times faster and contained 3.5 times more parsimony-informative sites than glyceraldehyde-3-phosphate dehydrogenase (gpd) sequences. Analyses of Alt a 1 gene and gpd exon sequences strongly supported grouping of Alternaria spp. and related taxa into several species-groups described in previous studies, especially the infectoria, alternata, porri, brassicicola, and radicina species-groups and the Embellisia group. The sonchi species-group was newly suggested in this study. Monophyly of the Nimbya group was moderately supported, and monophyly of the Ulocladium group was weakly supported. Relationships among species-groups and among closely related species of the same species-group were not fully resolved. However, higher resolution could be obtained using Alt a 1 sequences or a combined dataset than using gpd sequences alone. Despite high levels of variation in amino acid sequences, results of in silico prediction of protein secondary structure for Alt a 1 demonstrated a high degree of structural similarity for most of the species suggesting a conservation of function.


http://www.sciencedirect.com/science/article/B6WFV-49TRKD3-1/2/56463dc6d856569b5f84383b5d0457cb

We constructed and characterized a bacterial artificial chromosome (BAC) library for Epichlo" festucae, a genetically tractable fungal plant mutualist. The 6144 clone library with an average insert size of 87 kb represents at least 18-fold coverage of the 29 Mb genome. We used the library to assemble a 110 kb contig spanning the putative ornithine decarboxylase (odc) ortholog and subsequently expanded it to 228 kb with a single walking step in each direction. Furthermore, we evaluated conservation of microsynteny between E. festucae and some model filamentous fungi by comparing sequence available from a 43 kb region at the end of one BAC to publicly available fungal genome sequences. Orthologs to the 13 contiguous open reading frames (ORFs) identified in E. festucae are syntenic in Neurospora crassa and Magnaporthe grisea occurring in small sets of two, three or four colinear ORFs. This library is a valuable resource for research into traits important for the development and maintenance of a plant-fungus mutualistic symbiosis.

http://www.sciencedirect.com/science/article/B6WFV-49MDYXV-3/2/4b840452dc2cad662b694e6db23ee1fb

Different species of the lichen-forming ascomycete fungus Teloschistes were found to contain group IB introns at position S1506 in the small subunit ribosomal RNA gene. We have characterized the structural organization and phylogeny of the Teloschistes introns Tco.S1506, Tla.S1506, and Tvi.S1506. Common features to all the introns are a small size, a compact RNA structure, and an atypical catalytic ribozyme core sequence motif. Variations in intron sizes, due to sequence extensions in the P1 and P8 loop segments, were observed in different species and isolates. Phylogenetic analyses based on the ITS1-5.8S-ITS2 region as well as the introns show that the Teloschistes S1506 introns represent a distinct evolutionary isolated cluster among the nuclear group I introns. Furthermore, introns from different lineages of Teloschistes villosus appear not strictly vertically inherited probably due to horizontal transfer in one of the lineages.


http://www.sciencedirect.com/science/article/B6WFV-4C4WXRJ-1/2/ccdf2640e0859cbeade1089bae502fba

Species limits were investigated within the Fusarium graminearum clade (Fg clade) through phylogenetic analyses of DNA sequences from portions of 11 nuclear genes including the mating-type (MAT) locus. Nine phylogenetically distinct species were resolved within the Fg clade, and they all possess contiguous MAT1-1 and MAT1-2 idiomorphs consistent with a homothallic reproductive mode. In contrast, only one of the two MAT idiomorphs was found in five other species, four of which were putatively asexual, and the other was heterothallic. Molecular evolutionary analyses indicate the MAT genes are under strong purifying selection and that they are functionally constrained, even in species for which a sexual state is unknown. The phylogeny supports a monophyletic and apomorphic origin of homothallism within this clade. Morphological analyses demonstrate that a combination of conidial characters could be used to differentiate three species and three species pairs. Species rank is formally proposed for the eight unnamed species within the Fg clade using fixed nucleotide characters. Index Descriptors: Fusarium head blight; Mating-type; Histone H3; Homothallic; Heterothallic; Gene trees; Species trees; Species limits; Phylogeny; Reciprocal monophyly


http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-7/2/47512785770fe0db07710ac5a0f896d1
Guest is a transposable element of the Tc1/mariner superfamily with 30-40 bp terminal inverted repeats and a TA dinucleotide target site duplication. Guest was originally discovered in the St. Lawrence 74A laboratory strain of the filamentous fungus Neurospora crassa. In this report, Guest iterations subcloned from a cosmid library of the Oakridge 74A strain were used to design PCR primers that permitted the detection of Guest in wild isolates of N. crassa. Guest is present in N. crassa as multiple copies ranging between 100 bp and 2.4 kb and is present in the mating type locus of several Neurospora species. Bioinformatic analysis of the entire N. crassa genome (Oakridge 74A strain) detected 48 Guest iterations. All iterations appeared to have been inactivated either by repeat-induced point mutation or sequence deletion, with the majority being remnants less than 400 bp in length. The possible involvement of Guest in the evolution of the variable region that flanks the mating type idiomorphs in several Neurospora species is discussed.

Gene Expression Patterns  (3)


New techniques are being applied to identify all the genes involved in mammalian gonad development and differentiation. As this list of genes increases, understanding the potential interactions between these genes will become increasingly difficult. We used a real time reverse transcription PCR (real time RT-PCR) protocol to examine and compare the relative expression levels of 55 genes in individual mouse fetal gonads. Real time PCR analysis demonstrated that except for Sry, no differences in relative gene expression were detectable between XX and XY gonad/mesonephroi complexes at embryonic day (E)11.5. Following Sry peak expression at E11.5, a number of genes were expressed at significantly higher relative levels in E12-14 XY than XX gonads. Of six genes expressed at higher levels in E12.5-14 XX than XY gonads, three, Bmp2, Emx2, and Fgfr2, had not been reported previously. Our results caution that differential localization patterns observed with whole mount in situ hybridization techniques may not
accurately reflect changes in transcript levels. We conclude that real time PCR is an efficient and powerful tool for studying multiple gene expression patterns during gonad development and differentiation, and can provide insight into gene interactions.


http://www.sciencedirect.com/science/article/B6W8W-49201N7-1/2/d890152e622144d4d2bb32a8a514c

Frizzleds are transmembrane receptors that can transduce signals dependent upon binding of Wnts, a large family of secreted glycoproteins homologous to the Drosophila wingless (wg) gene product and critical for a wide variety of normal and pathological developmental processes. In the nervous system, Wnts and Frizzleds play an important role in anterior-posterior patterning, cell fate decisions, proliferation, and synaptogenesis. However, little is known about the role of Frizzled signaling in the developing eye. We isolated cDNAs for ten chick Frizzleds and analyzed the spatial and temporal expression patterns during eye development in the chick embryo. Frizzled-1 to -9 are specifically expressed in the eye at various stages of development and show a complex and partially overlapping pattern of expression.


http://www.sciencedirect.com/science/article/B6W8W-47P8V78-1/2/3cac6568bf37c52785687f811299c1e3

The only molecular similarity shown so far for sexual regulatory genes among different phyla involves doublesex (dsx) of Drosophila, mab-3 and mab-23 of Caenorhabditis elegans, and Dmrt1 of vertebrates. These genes encode DM domain transcription factors (DM=dsx and mab-3) and are required for sexual differentiation. In the case of dsx and mab-3, the two genes control analogous aspects of sexual development, bind similar DNA sequences, and are capable of functional substitution in vivo. All three phyla have multiple DM domain genes, but it is unknown how many of these are involved in sexual development. Mammals, for example, have at least seven DM domain genes, but embryonic expression has only been examined in detail for Dmrt1 (dsx- and mab-3 related transcription factor 1). We have identified additional murine DM domain genes and have examined their expression in the mouse embryo, with emphasis on the developing gonad. At least three murine DM domain genes in addition to Dmrt1 are expressed in the embryonic gonad: Dmrt4 is expressed at similar levels in gonads of both sexes; Dmrt3 is more highly expressed in males; and Dmrt7 is more highly expressed in females. Expression of three other genes is low or absent in the embryonic gonad. Two of these, Dmrt5 and Dmrt6, are expressed primarily in the brain, and the third, Dmrt2, is expressed in presomitic mesoderm and developing somites. Our data suggest that multiple DM domain genes may be involved in mammalian sexual development, and that they may function in both testis and ovary development.

http://www.sciencedirect.com/science/article/B6WG0-4BVP3YD-4/2/f43e453de5af4e4ad0d86fac1d3c085

We analyzed the genes that exhibit transcriptional changes during sex differentiation in Xenopus, using fluorescent differential display (FDD). Search was then undertaken for sequences that were homologous to the differentially displayed DNA. In this report, trans-acting factors of activating transcription factor 4 (ATF 4) and heat shock proteins were selected, on the basis of homology, from candidate genes thought to be involved in the expression cascade of aromatase and estrogen receptor genes. The stage and tissue specificities and the effect of estradiol treatment on the expression of these genes were then examined using real-time quantitative polymerase chain reaction (RQ-RT-PCR). The expression of ATF 4, a member of the ATF/cAMP-responsive element-binding protein (CREB) family of genes, peaked in the gonads at stage 50 of development. Interestingly, expression of the genes encoding the heat shock cognate protein70. II (Hsc70. II) and the heat shock protein 70 (Hsp70) binding protein was strongly activated at stages 50 and 48 of development, respectively. The three genes revealed a higher transcription activity in the gonads than in other tissues. Although the expression of all of the genes encoding ATF 4, aromatase, Hsc70. II, and Hsp70 binding protein was activated in vitro by estrogen treatment, that of Hsc70. II and Hsp70 binding protein was found to be transient.


http://www.sciencedirect.com/science/article/B6WG0-4C47KS6-3/2/8d0892c250c58a224cf2884ad86977d8

Effects of salmon gonadotropin-releasing hormone (sGnRH) and estradiol-17[beta] (E2) on gene expression and release of gonadotropins (GTHs) were examined in masu salmon (Oncorhynchus masou) using primary pituitary cell cultures at three reproductive stages, initiation of sexual maturation in May, pre-spawning in July, and spawning in September. Amounts of GTH subunit mRNAs were determined by real-time polymerase chain reaction, and levels of GTH released in the medium were determined by RIA. In control cells, the amounts of three GTH subunit mRNAs ([alpha]2, FSH[beta], and LH[beta]) peaked in July prior to spawning. FSH release spontaneously increased with gonadal maturation and peaked in September, whereas LH release remained low until July and extensively increased in September. Addition of E2 to the culture extensively increased the amounts of LH[beta] mRNA in May and July in both sexes. It also increased the [alpha]2 mRNA in July in the females. In contrast, sGnRH alone did not have any significant effects on the amounts of three GTH subunit mRNAs at all stages, except for the elevation of [alpha]2 and FSH[beta] mRNAs in July in the females. Nevertheless, synergistic effects by sGnRH and E2 were evident for all three GTH subunit mRNAs. In May, sGnRH in combination with E2 synergistically increased the amounts of LH[beta] mRNA in the males and [alpha]2 mRNA in the females. However, in July the combination suppressed the amounts of [alpha]2 and FSH[beta] mRNAs in the females. sGnRH alone stimulated LH release at all stages in both sexes, and the release was synergistically enhanced by E2. Synergistic stimulation of FSH release was also observed in May and July in both sexes. These results indicate that a functional interaction of sGnRH with E2 is differently involved in synthesis and release of GTH. The synergistic interaction modulates GTH synthesis differentially, depending on subunit, stage, and gender, whereas it potentiates the activity of GnRH to release GTH in any situation.

http://www.sciencedirect.com/science/article/B6WG0-4C604P9-2/2/716494e76c66da69c2e35bad5a185de

We developed a one-tube two-temperature real-time RT-PCR that allows to absolutely quantify the gene expression of hormones using the standard curve method. As our research focuses on the expression of the insulin-like growth factors (IGFs) in bony fish, we established the technique for IGF-I and IGF-II using the tilapia (Oreochromis niloticus) as model species. As approach, we used primer extension adding a T7 phage polymerase promoter (21 nt) to the 5'end of the antisense primers. This procedure avoids the disadvantages arising from plasmids. Total RNA extracted from liver was subjected to conventional RT-PCR to create templates for in vitro transcription of IGF-I and IGF-II cRNA. Correct template sizes including the T7 promoter were verified (IGF-I: 91 nt; IGF-II: 94 nt). The PCR products were used to create IGF-I and IGF-II cRNAs which were quantified in dot blot by comparison with defined amounts of standardised kanamycin mRNA. Standardised threshold cycle (Ct) values for IGF-I and IGF-II mRNA were achieved by real-time RT-PCR and used to create standard curves. To allow sample normalisation the standard curve was also established for [beta]-actin as internal calibrator (template: 86 nt), and validation experiments were performed demonstrating similar amplification efficiencies for target and reference genes. Based on the standard curves, the absolute amounts of IGF-I and IGF-II mRNA were determined for liver (IGF-I: 8.90 +/- 1.90 pg/[mu]g total RNA, IGF-II: 3.59 +/- 0.98 pg/[mu]g total RNA) and extrahepatic sites, such as heart, kidney, intestine, spleen, gills, gonad, and brain considering the different lengths of cRNAs and mRNAs by correction factors. The reliability of the method was confirmed in additional experiments. The amplification of descending dilutions of cRNA and total liver RNA resulted in parallel slopes of the amplification curves. Furthermore, amplification plots of the standard cRNA and the IGF-I and IGF-II mRNAs showed signals starting at the expected Ct values. Thus, the one-tube RT-PCR described here is highly sensitive (detection level ~2 pg/[mu]g total RNA) and allows precise absolute quantification. The method is rapid as there are neither separate reverse transcriptions nor post-amplification steps, and can be executed with low risk of contamination. Therefore, it will be helpful when investigating gene expression in any species and tissue whenever absolute levels are of concern.


http://www.sciencedirect.com/science/article/B6WG0-48DXR9M-1/2/6d29ab09d9d5967b65096339d6c1d67a

Fish endure long periods of fasting and demonstrate an extensive capacity for rapid and complete recovery after refeeding. The underlying mechanisms through which nutrient intake activates an increase in somatic growth and especially in muscle growth is poorly understood. In this study we examined the expression profile of major muscle growth regulators in trout white muscle 4, 12, and 34 days after refeeding, using real-time quantitative RT-PCR. Mean insulin-like growth factor I (IGFI) mRNA level in muscle increased dramatically 8- and 15-fold, 4 and 12 days, respectively, after refeeding compared to fasted trout. This declined thereafter. Conversely, only a weak but gradual increase in mean insulin-like growth factor II (IGFII) mRNA level was observed during refeeding. Inversely to IGFI, mean IGF receptor Ia (IGFRIa) mRNA level declined after ingestion of food. In contrast, IGF receptor Ib (IGFRIb) mRNA level was not affected by refeeding. Mean
fibroblast growth factor 2 (FGF2) mRNA level increased by 2.5-fold both 4 and 12 days after refeeding, whereas fibroblast growth factor 6 (FGF6) and myostatin mRNA levels were unchanged. Subsequent to IGF1 and FGF2 gene activation, an increase in myogenin mRNA accumulation was observed at 12 days post-refeeding suggesting that an active differentiation of myogenic cells succeeds their proliferation. In conclusion, among the potential growth factors we examined in this study, IGF1 and FGF2 were identified as candidate genes whose expression may contribute to muscle compensatory growth induced by refeeding.


Previous studies have demonstrated that FSH stimulates cell proliferation in the ovary and the testis of the chick embryo. This study analyzed the presence of FSH receptor and the cell subpopulations that proliferate in response to FSH in chick embryo gonads. FSH receptor mRNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in the male and female gonads of the 6 to 14-day-old chick embryo. Somatic cells of the ovary expressed the FSH receptor in the 14-day-old chick embryo. Ovarian surface epithelium of the 14-day-old chick embryo increased the mitotic index 15-18 h after FSH treatment. Similarly, the mitotic index in oogonia was increased 24 h after receiving a pulse of FSH; this result was confirmed by an increase in the number of germ cells that incorporated bromodeoxyuridine (BrdU). Somatic cells of the medullary cords in the ovary displayed an increase in the mitotic index 15-21 h after the FSH injection. In the chick embryo testis, at the same stage of development, the treatment with FSH increased the mitotic index in cells of the seminiferous tubules and to a lesser extent in cells at a peritubular and interstitial location. Present results demonstrate that in the chick embryo, FSH stimulates the proliferation of ovarian surface epithelium, oogonia in the cortex, and somatic cells of the medullary cords of the chick embryo ovary. In the chick embryo testis, FSH stimulates cell proliferation in seminiferous tubules and peritubular cells.


It has been hypothesized that estrogen production may play a pivotal role in the sex determination of reptiles with temperature-dependent sex determination (TSD). This hypothesis has been furthered by studies that have shown higher aromatase activity in the developing ovaries in some reptiles. However, other studies have not consistently supported this hypothesis. In the current study we addressed this issue by cloning P450 aromatase cDNA in the turtle,
Trachemys scripta, and developing a quantitative competitive RT-PCR for aromatase. This assay was then used to quantify aromatase mRNA levels in adrenal-kidney-gonad complexes (AKG) during TSD. Aromatase mRNA was detected in the AKGs at both male- and female-producing temperatures from the earliest stage of development sampled (stage 15), through hatching (stage 26). However, levels remained relatively constant during the thermosensitive period of TSD. Further, no significant difference was detected between male- and female-producing temperatures during the thermosensitive period. After the thermosensitive period, aromatase mRNA levels increased in females (this coincides with the period during which the ovaries are differentiating). These results are consistent with those of several previous studies of certain reptiles with TSD. The current results suggest that the expression of aromatase may not be a pivotal regulatory step in the sex determination cascade of this turtle.


In the present study we cloned, sequenced, and confirmed the presence of mRNAs of gonadotropins (FSH-[beta], LH-[beta] subunits) from the brain and pituitary of tilapia, Oreochromis niloticus. Further, we examined the spatio-temporal expression pattern of FSH-[beta] and LH-[beta] in the brain and pituitary of two species of teleost (tilapia, O. niloticus; sockeye salmon, Oncorhynchus nerka), using in situ hybridization and immunological methods. The expression of FSH and LH immunoreactivity appeared simultaneously in the brain and pituitary (tilapia, 14 days; sockeye, 51 days after fertilization). In the pituitary, FSH mRNA and peptide expressing cells were distinct from LH expressing cells located in the ventral proximal pars distalis. In the brain, FSH and LH immunoreactivity was co-localized in cells of the preoptic nucleus parvocellularis, magnocellularis, and gigantocellularis. Fibers immunoreactive to FSH and LH antisera were seen along the forebrain-hypothalamus and in the neurohypophysis of the pituitary. Double-label immunofluorescence revealed FSH and LH immunoreactivity co-localized in arginine vasotocin synthesizing preoptic neurons. Our results show that FSH and LH-producing cells have developmental origins in the brain as well as in the pituitary. In addition, we propose that the brain-derived gonadotropins may function as hypophysiotropic hormones that regulate pituitary cells and along with arginine vasotocin could act as neuromodulators of reproductive behaviors.


The hormone insulin-like growth factor-I (IGF-I) regulates vertebrate growth. The liver produces most circulating IGF-I, under the control of pituitary growth hormone (GH) and nutritional status. To study the regulation of liver IGF-I production in salmon, we established a primary hepatocyte culture system and developed a TaqMan quantitative real-time RT-PCR assay for salmon IGF-I gene expression. A portion of the coho salmon acidic ribosomal phosphoprotein P0 (ARP) cDNA was sequenced for use as a reference gene. A systematic bias across the 96 well PCR plate was discovered in an initial IGF-I assay, which was corrected when the assay was redesigned. IGF-I mRNA levels measured with the validated assay correlated well with levels measured with an RNase protection assay, and were highest in liver compared with other tissues. We examined the
time course of hepatocyte IGF-I gene expression over 48 h in culture, the response to a range of
GH concentrations in hepatocytes from fed and fasted fish, and potential effects of variation in
IGF-I in the medium. IGF-I gene expression decreased over time in culture in hepatocytes in plain
medium, and in cells treated with 5 nM GH with or without a combination of metabolic hormones
(1 [μM insulin, 100 nM triiodothyronine, and 0.1 nM dexamethasone). GH stimulated IGF-I
gene expression at all time points. In cells treated with GH plus metabolic hormones, IGF-I gene
expression was intermediate between the controls and GH alone. Increasing concentrations of
GH resulted in biphasic IGF-I gene expression response curves in cells from fed and fasted fish,
with the threshold for stimulation from 0.5 to 2.5 nM GH, maximal response from 5 to 50 nM, and
a reduced response at 500 nM. Medium IGF-I (5 nM) did not affect basal or GHstimulated IGF-I
gene expression. This study shows that primary hepatocyte culture and the TaqMan IGF-I assay
can be used to study the regulation of hepatic IGF-I gene expression in salmon, and provides the
first evidence of a biphasic response to GH concentration in fish hepatocyte culture.

acetyltransferase and melatonin receptor genes in the eye and brain of chum salmon
(Oncorhynchus keta)." General and Comparative Endocrinology 136(3): 311.

http://www.sciencedirect.com/science/article/B6WG0-4BRSDS1-2/2/8e506faffa6dfe980e09121e30e40565c

Melatonin and arylalkylamine N-acetyltransferase (AANAT), the rate-limiting enzyme in melatonin
synthesis, have taken on special importance in vertebrate circadian biology. Recent identification
of genes encoding two AANAT (AANAT1 and AANAT2) and two subtypes of melatonin receptor
(Mel-R; Mel1a and Mel1b) in several fish species has led to rapid advances in characterizing the
physiological roles of melatonin. In the present study, partial cDNAs encoding these four genes
were cloned from the eye and brain of chum salmon (Oncorhynchus keta). Based on the
nucleotide sequences, we developed highly sensitive real-time PCR systems for these four
mRNAs. The development of daily rhythmicity in AANAT1, AANAT2, Mel1a, and Mel1b transcript
levels was examined in the eye and brain of chum salmon during embryonic and post-embryonic
stages (from day -9 to day +180). In a parallel experiment, ocular and brain melatonin levels were
measured by radioimmunoassay. Parallelism in developmental changes and in circadian rhythms
of AANAT mRNAs and melatonin levels in the eye and the brain supports a hypothesis that the
developmental increases of nocturnal melatonin levels results partly from the elevated
transcription of AANAT genes. Moreover, abundant expression of AANAT and Mel-R mRNAs in
the optic tectum, thalamus, hypothalamus, cerebellum, and eye indicates possible roles of
melatonin in visual processing and neuroendocrine regulation, through which melatonin might be
involved in migratory behavior of chum salmon.

Slagter, B. J., J. D. Kittilson, et al. (2004). "Somatostatin receptor subtype 1 and subtype 2 mRNA
expression is regulated by nutritional state in rainbow trout (Oncorhynchus mykiss)." General and
Comparative Endocrinology 139(3): 236.

http://www.sciencedirect.com/science/article/B6WG0-4DPGX17-2/2/22470cf38983b4aa9d630c0452728941

Somatostatin receptors (sst) mediate the numerous physiological actions (e.g., aspects of growth,
development, and metabolism) of the somatostatin family of peptides. In this study, we used
rainbow trout (Oncorhynchus mykiss) to establish the pattern of sst subtype 1A, 1B, and 2 mRNA
expression in selected tissues (optic tectum of brain, endocrine pancreas, and liver) and to
evaluate nutritional regulation of sst expression. Quantitative real-time reverse transcription-PCR,
sensitive to less than 100 copies, revealed that sst1s and sst2 was differentially expressed, both
in terms of distribution among the tissues of study and in terms of relative abundance within a particular tissue. Under normal physiological (fed) conditions, sst1B levels were two times greater than those of sst1A in all tissues examined and levels of sst2 were 2-5 times greater those of sst1B, except in optic tectum, in which sst1B and sst2 mRNA levels appeared equal. Nutritional state modulated the pattern of sst1 and sst2 mRNAs expression. Fasting for 2 or 6 weeks reduced the expression of sst mRNAs in optic tectum; whereas, fasting increased the expression of sst mRNAs in both pancreas and liver. Re-feeding animals for 2 weeks following a 4-week fast restored mRNA levels to near those in tissues from animals which were fed continuously. These findings indicate that the pattern of sst expression in optic tectum, pancreas, and liver is regulated by nutritional state.


http://www.sciencedirect.com/science/article/B6WG0-49R5F1V-1/2/888f5dbf0166866daad2c7e01eb37afc

Proopiomelanocortin (POMC) is a precursor for several pituitary hormones including adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH) and endorphin (END). Fish POMCs in four taxonomic classes, Cephalaspidomorphi (lampreys), Chondrichthyes (cartilaginous fish), Sarcopterygii (lobe-finned fish), and Actinopterygii (ray-finned fish) have been identified. However, two essential species, ratfish in Chondrichthyes and hagfish in Agnatha, are still missing in the evolutionary image of this molecule. The present study reports analysis of POMC cDNA in the ratfish, Chimaera phantasma, which belongs to another subclass in the Chondrichthyes. Partial cDNA clones were amplified by PCR from single-strand cDNA prepared on total RNA from a complex of pituitary and hypothalamus, and subsequently overlapped to obtain a full-length sequence. Ratfish POMC cDNA consists of 1294 bp excluding the poly(A) tail. It encodes a signal peptide of 25 amino acids and POMC of 300 amino acids. [gamma]-MSH, ACTH, [alpha]-MSH, [delta]-MSH, [beta]-MSH, and [beta]-END are located at prePOMC (76-87), (120-158), (120-132), (212-227), (275-290), and (293-325), respectively. [delta]-MSH, originally found in elasmobranch POMCs, was also present in ratfish POMC, suggesting this structure might have appeared after the divergence of chondrichthians from the ancestral lineage. Thus, we demonstrated the common occurrence of four MSHs in chondrichthian POMC and established a clear understanding of the molecular evolution of POMC in gnathostomes.

http://www.sciencedirect.com/science/article/B6WG0-4FH5K4K-2/2/2de9d1a70575a7ad6738188dbae4fa1d

Extrapituitary expression of the growth hormone (GH) gene has been reported for the immune system of various vertebrates. In the rainbow trout (Oncorhynchus mykiss), GH mRNA could be detected in several lymphoid organs and leucocytes by reverse transcriptase-polymerase chain reaction (RT-PCR). To understand the control of GH expression in the fish immune system, mRNA levels for two distinct GH genes (GH1 and GH2) in trout leucocytes isolated from peripheral blood were quantified using a real-time PCR method. Both GH mRNAs could be detected in trout leucocytes, although their levels were extremely low compared to those in pituitary cells. The levels of GH2 mRNA in leucocytes were several times higher than those of GH1, while no difference was observed between GH1 and GH2 mRNA levels in the pituitary. Administration of dibutyryl cyclic AMP and cortisol produced a significant elevation of GH mRNA levels in trout leucocytes, although the levels were unchanged by T3. GH1 and GH2 mRNA levels showed similarities in responses to those factors. The effect of cortisol on GH mRNA appears
biphasic; a dose-depending elevation of GH gene expression was observed in leucocytes treated with cortisol at below 200 nM, however, cortisol had no effect at 2000 nM. Cortisol-treated leucocytes showed no significant change in the mRNA level of [beta]-actin or proliferative activity during the experiments. Our results thus show that, at the low levels, GH gene expression in trout leucocytes is regulated by cortisol, which has been known as a regulatory factor of GH gene expression in pituitary cells, and suggest a physiological significance of paracrine GH produced in the fish immune system.

**General Pharmacology**


http://www.sciencedirect.com/science/article/B6T71-413KW4H-4/2/509d36a1a34ff94d6b1ae7ac2a48388d

Catecholamines induce direct vasoconstriction mediated by postsynaptic [alpha]-adrenergic receptors ([alpha]-ARs) of both the [alpha]1 and [alpha]2 type. To evaluate the contribution of each [alpha]2-AR subtype ([alpha]2A, [alpha]2B, and [alpha]2C) to this function, we used groups of genetically engineered mice deficient for the gene to each one of these subtypes and compared their blood pressure (BP) responses to their wild-type counterparts. Blood pressure responses to a bolus of norepinephrine (NE) were assessed before and after sequential blockade of [alpha]1-ARs with prazosin and [alpha]2-ARs with yohimbine. The first NE bolus elicited a brief 32 to 44 mm Hg BP rise (p 2A-AR gene knockouts differed, responding instead with a 20-mm Hg fall in BP, a significant change from baseline (p 2A-AR knockouts. Norepinephrine bolus during concurrent [alpha]1 and [alpha]2-AR blockade produced significant (p 2-vascular wall ARs. We conclude that the [alpha]2-AR-mediated vasoconstriction induced by catecholamines is attributable to the [alpha]2A-AR subtype because mice deficient in any one of the other subtypes retained the capacity for normal vasoconstrictive responses. However, the [alpha]1-ARs account for the major part (as much as 68%) of catecholamine-induced vasoconstriction.

**General Pharmacology: The Vascular System**


http://www.sciencedirect.com/science/article/B6T71-44CMG4M-3/2/c2e4333372fc90551c828318dc76a688

Our earlier observations suggest that M3 muscarinic acetylcholine (ACh) receptors (mACHRs) are involved in Ca2+ signaling and regulation of c-fos gene expression in T lymphocytes. Here, we describe the effects of YM905, a novel M3 antagonist, on evoked Ca2+ signaling and c-fos gene
expression in CEM human leukemic T cells. YM905 significantly inhibited increases in intracellular free Ca\textsuperscript{2+} evoked by 10 [\mu]M oxotremorine-M, an M1/M3 agonist (IC\textsubscript{50}=100 nM), and also inhibited 10 [\mu]M oxotremorine-M-induced upregulation of c-fos gene expression at 1 [\mu]M. These findings demonstrate that YM905 antagonizes the intracellular responses in T cells induced via mAChRs, possibly M3 receptors.

Genes & Dev. (2)


http://www.genesdev.org/cgi/content/abstract/18/21/2652

Nuclear export of mRNA is a central step in gene expression that shows extensive coupling to transcription and transcript processing. However, little is known about the fate of mRNA and its export under conditions that damage the DNA template and RNA itself. Here we report the discovery of four new factors required for mRNA export through a screen of all annotated nonessential Saccharomyces cerevisiae genes. Two of these factors, mRNA surveillance factor Rrp6 and DNA repair protein Lrp1, are nuclear exosome components that physically interact with one another. We find that Lrp1 mediates specific mRNA degradation upon DNA-damaging UV irradiation as well as general mRNA degradation. Lrp1 requires Rrp6 for genomic localization to genes encoding its mRNA targets, and Rrp6 genomic localization in turn correlates with transcription. Further, Rrp6 and Lrp1 are both required for repair of UV-induced DNA damage. These results demonstrate coupling of mRNA surveillance to mRNA export and suggest specificity of the RNA surveillance machinery for different transcript populations. Broadly, these findings link DNA and RNA surveillance to mRNA export.

Sawado, T., J. Halow, et al. (2003). "The \beta\textsuperscript-\text{-globin locus control region (LCR) functions primarily by enhancing the transition from transcription initiation to elongation." Genes & Dev. 17(8): 1009-1018.

http://www.genesdev.org/cgi/content/abstract/17/8/1009

To investigate the molecular basis of [\beta\text{-}globin gene activation, we analyzed factor recruitment and histone modification at the adult [\beta\text{-}globin gene in wild-type (WT)/locus control region knockout ([\Delta\text{LCR}) heterozygous mice and in murine erythroleukemia (MEL) cells. Although histone acetylation and methylation (Lys 4) are high before and after MEL differentiation, recruitment of the erythroid-specific activator NF-E2 to the promoter and preinitiation complex (PIC) assembly occur only after differentiation. We reported previously that targeted deletion of the LCR reduces [\beta\text{-}globin gene expression to 1%-4% of WT without affecting promoter histone acetylation. Here, we report that NF-E2 is recruited equally efficiently to the adult [\beta\text{-}globin promoters of the [\Delta\text{LCR and WT alleles. Moreover, the LCR deletion reduces PIC assembly only twofold, but has a dramatic effect on Ser 5 phosphorylation of RNA polymerase II and transcripational elongation. Our results suggest at least three distinct stages in [\beta\text{-}globin gene activation: (1) an LCR-independent chromatin opening stage prior to NF-E2 recruitment to the promoter and PIC assembly; (2) an intermediate stage in which NF-E2 binding (LCR-independent) and PIC assembly (partially LCR-dependent) occur; and (3) an LCR-dependent fully
active stage characterized by efficient pol II elongation. Thus, in its native location the LCR functions primarily downstream of activator recruitment and PIC assembly.

**Genes Cells** (5)


http://www.genestocellsonline.org/cgi/content/abstract/10/3/207

The general transcription factor TFIIE plays essential roles in transcription by RNA polymerase II (PolII). Despite recent progress, the elucidation of its precise mechanisms including biological functions awaits further characterization. We report the isolation and characterization of Schizosaccharomyces pombe TFIIE (spTFIIE). Like human and other eukaryotic TFIIE proteins, spTFIIE consists of {alpha} and {beta} subunits and the genes encoding both subunits are essential for viability. Chromatin immunoprecipitation assays demonstrated that spTFIIE localizes to promoters in vivo. Mutational analysis of the C-terminal basic helix-loop region of TFIIE{beta}, which is involved in the transition from transcription initiation to elongation, revealed that transcription-defective mutants affected in this region are also cold sensitive. The spTFIIE{beta} subunit binds both spTFIIE{beta} and spTFIIE{alpha} but spTFIIE{alpha} binds only spTFIIE{beta}. These results indicate that TFIIE forms an {alpha}2{beta}2 heterotetramer in which two {alpha}{beta} heterodimers are connected via {beta} subunits. Further analysis of binding specificities showed that spTFIIE{beta} binds the Rpb2 and Rpb12 subunits of PolII, whereas spTFIIE{alpha} predominantly binds Rpb5, which is located at the clamp region and changes conformation upon transcription initiation.


http://www.genestocellsonline.org/cgi/content/abstract/9/10/905

We previously reported that DNA topoisomerase II (topo II) is required for the G0-to-S phase transition in mammalian cells [Hossain et al. (2002) ICRF-193, a catalytic inhibitor of DNA topoisomerase II, inhibits re-entry into the cell division cycle from quiescent state in mammalian cells. Genes Cells 7, 285-294]. In this study, we examined whether the requirement for topo II is evolutionarily conserved in Drosophila and yeast. ICRF-193, a catalytic inhibitor of topo II, inhibited DNA synthesis in Drosophila Schneider cells released from the G0 (stationary) phase, whereas the drug did not inhibit DNA synthesis in Schneider cells released from the M phase. Depletion of topo II mRNA by RNA-interference (RNAi) in G0-phase Schneider cells resulted in significant inhibition of DNA synthesis after release from G0-arrest. In the yeast topo II temperature-sensitive (ts) mutant, the initial cycle of DNA synthesis occurred at a restrictive temperature after release from starvation-induced G0 phase and doubling of the DNA content in the cells was confirmed by both flow cytometry and fluorescence spectrophotometry. DNA synthesis in yeast cells after release from the G0 phase was also observed in the presence of ICRF-193. Doubling of the DNA content was observed during spore germination of topo II ts mutant yeast at a restrictive temperature as determined by fluorescence spectrophotometry.
These results indicate that topo II is required for the G0-to-S phase transition in Drosophila Schneider cells, but not in yeast.


http://www.genestocellsonline.org/cgi/content/abstract/9/3/253

DNA methylation controls various developmental processes by silencing, switching and stabilizing genes as well as remodeling chromatin. Among various symptoms in cloned animals, placental hypertrophy is commonly observed. We identified the Spalt-like gene3 (Sall3) locus as a hypermethylated region in the placental genome of cloned mice. The Sall3 locus has a CpG island containing a tissue-dependent differentially methylated region (T-DMR) specific to the trophoblast cell lineage. The T-DMR sequence is also conserved in the human genome at the SALL3 locus of chromosome 18q23, which has been suggested to be involved in the 18q deletion syndrome. Intriguingly, larger placentas were more heavily methylated at the Sall3 locus in cloned mice. This epigenetic error was found in all cloned mice examined regardless of sex, mouse strain and the type of donor cells. In contrast, the placentas of in vitro fertilized (IVF) and intracytoplasmic sperm injected (ICSI) mice did not show such hypermethylation, suggesting that aberrant hypermethylation at the Sall3 locus is associated with abnormal placental development caused by nuclear transfer of somatic cells. We concluded that the Sall3 locus is the area with frequent epigenetic errors in cloned mice. These data suggest that there exists at least genetic locus that is highly susceptible to epigenetic error caused by nuclear transfer.


http://www.genestocellsonline.org/cgi/content/abstract/7/5/497

BACKGROUND: The sex hormone 17beta-oestradiol (E2) has profound effects on many aspects of reproduction, development, as well as behaviour. Although the oestrogen receptor is well characterized on a molecular level, relatively few genes affected by E2 have been identified, and the mechanisms underlying the physiological changes caused by E2 are largely unknown. In order to identify oestrogen-regulated genes in vivo, early uterine gene expression profiles were developed using DNA microarrays. RESULTS: Ovariectomized mice were exposed to 17beta-oestradiol for 6 h, and mRNA expression analysis for 9977 genes was performed. Although a large number of genes was affected by oestrogen administration, the genes that showed higher reproducibility in repetitive experiments were selected and further examined. For most of the selected genes, expression was induced in a dose-dependent manner, and gene expression was not altered following oestrogen treatment in oestrogen receptor-alpha (ER(alpha))-deficient mice. In combination with the estimation of gene expression levels using quantitative PCR, it was revealed that multiple genes related to sterol biosynthesis, tRNA synthesis, RNA processing, and growth signalling were activated. Based on the microarray data, we selected additional genes related to sterol biosynthesis and tRNA synthesis and confirmed that these genes are also activated by oestrogen. CONCLUSION: Genes suggesting a basis for the drastic uterotrophic effect observed several days following oestrogen administration were identified. These findings not only reveal the diverse effect of oestrogen signalling on transcript levels in vivo but also demonstrate the ability of DNA microarrays to identify cellular pathways affected by oestrogen.

http://www.genestocellsonline.org/cgi/content/abstract/8/4/403

BACKGROUND: BAT1 belongs to the DEAD-box family of proteins, and is encoded in the central region of the MHC, a region containing genes affecting immunopathological disorders including Type 1 diabetes. We showed that BAT1 can reduce inflammatory cytokine production, supporting its candidacy as a disease gene. Here we examined the proximal promoter region of BAT1.

RESULTS: Ten single nucleotide polymorphisms were identified in approximately 1.4 kb of sequence, defining at least seven alleles. Sections of the BAT1 promoter region were amplified from cells homozygous for the MHC haplotypes associated with susceptibility (HLA-A1, B8, DR3; 8.1 haplotype) and resistance (HLA-A3, B7, DR15; 7.1 haplotype) to diabetes and cloned into a promoter-less luciferase-encoding plasmid. Jurkat cells transiently transfected with fragments from the 8.1 haplotype exhibited a lower luciferase activity than those transfected with fragments from the 7.1 haplotype, indicating reduced transcription. The effect was clearest with the 520 bp immediately upstream of the transcriptional start site. Electrophoretic mobility shift assays using oligonucleotides spanning polymorphic sites within the 520 bp (proximal) promoter fragment showed haplotype-specific binding of nuclear proteins.

CONCLUSIONS: In view of the anti-inflammatory role of BAT1, reduced production on a disease-associated haplotype constitutes a novel and self-consistent model for the effect of central MHC genes on disease.

Genetic Analysis: Biomolecular Engineering  (18)


http://www.sciencedirect.com/science/article/B6T72-3Y15VD3-5/2/400c9981dec8f66933dd5e5383367723

We report a reliable method for PCR (polymerase chain reaction) amplification of genomic DNA from PET. This method uses DNA extraction with the QIAquick kit and amplification with AmpliTaq Gold. Amplification of up to 959 bp from PET was achieved with this combination which exceeds the current reported upper limit of 800 bp. In summary, the gradual activation of the AmpliTaq Gold during thermal cycling allows both for higher-fidelity and higher-throughput PCR amplification from PET. The use of the QIAquick kit for DNA purification of PET is sensitive, reproducible and suitable for management of a high number of samples.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2N/2/4b5e1ef5c1593ebe95a2d01ac40679f9

Beginning with 103-105 molecules of a purified HIV-1 target sequence as a starting template, we
have examined the effects of starting template concentration and cycle number on the amplification efficiency of the polymerase chain reaction. An external standard DNA sequence has been designed that when added to a DNA sample enables a determination of the starting concentration of HIV-1 target sequence in that sample of DNA. Varying ratios of external standard and target DNA sequences were amplified for 22 cycles. When the starting concentration of the external standard was within 50-fold of the starting concentration of the target, the amplifications of both sequences were proportional. These same results were obtained when the two templates were amplified in the presence of an excess of heterogeneous genomic DNA. Using this quantitative method, the number of starting target molecules in a DNA sample can be calculated to within a two-fold range of accuracy.


http://www.sciencedirect.com/science/article/B6T72-4840KWT-T/2/1a951e2b6a250763b3a5e93e5bad9688

Physical mapping of the human genome involves a variety of complex hybridization-based procedures, some of which rely upon the ability to separate human clones derived from human-rodent hybrid cell lines from those that contain background rodent-derived DNA sequences. The ability to block the repetitive element (Alu repeat) portion of inter-Alu PCR products derived from a variety of complex sources is also crucial for the isolation of unique DNA sequences. Here we report the construction and characterization of a new consensus Alu repeat probe (pPD39) designed for these purposes.


http://www.sciencedirect.com/science/article/B6T72-3VW821K-4/2/9327b4d3b07a553d158e493490a8ced5

We previously described a targeted genomic differential display method (TGDD; Broude NE, Chandra A, Smith CL. Differential display of genomic subsets containing specific interspersed repeats. Proc. Natl. Acad. Sci. USA 1997;94:4548-53). In that method, presently characterized as method I, targeting was accomplished by capturing DNA fragments containing specific a sequence by hybridization with complementary single-stranded DNA. The captured fragments were amplified by PCR. Here, we describe method II where targeting is accomplished by PCR using primers specific to the target sequence. Method II takes advantage of PCR suppression to eliminate fragments not containing the target sequence (Siebert PDA, Chenchik A, Kellogg DE, Lukyanov KA and Lukyanov SA. An improved PCR method for walking in uncloned genomic DNA. Nucleic Acids Res 1995;23:1087-1088). Targeting focuses analysis on and around interesting areas and additionally serves to reduce the complexity of the amplified subset. These approaches are useful to amplify genome subsets containing a variety of targets including various conserved sequences coding for cis-acting elements or protein motifs.

A homogeneous detection mechanism based on fluorescence resonance energy transfer (FRET) has been developed for two DNA diagnostic tests. In the template-directed dye-terminator incorporation (TDI) assay, a donor dye-labeled primer is extended by DNA polymerase using allele-specific, acceptor dye-labeled ddNTPs. In the dye-labeled oligonucleotide ligation (DOL) assay, a donor dye-labeled common probe is joined to an allele-specific, acceptor dye-labeled probe by DNA ligase. Once the donor and acceptor dyes become part of a new molecule, intramolecular FRET is observed over background intermolecular FRET. The rise in FRET, therefore, can be used as an index for allele-specific ddNTP incorporation or probe ligation. Real time monitoring of FRET greatly increases the sensitivity and reliability of these assays. Change in FRET can also be measured by end-point reading when appropriate controls are included in the experiment. FRET detection proves to be a robust method in homogeneous DNA diagnostic assays.


Important requirements for molecular genetic epidemiological studies are economy, sample parallelism, convenience of setup and accessibility, goals inadequately met by existent approaches. We invented microplate array diagonal gel electrophoresis (MADGE) to gain simultaneously the advantages of simple setup, 96-well microplate compatibility, horizontal electrophoresis, and the resolution of polyacrylamide. At essentially no equipment cost (one simple plastic gel former), 10-100-fold savings on time for sample coding, liquid transfers, and data documentation, in addition to volume reductions and gel re-use, can be achieved. MADGE is compatible with ARMS, restriction analysis and other pattern analyses. CpG-PCR is a general PCR approach to CpG sites (10-20% of all human single base variation): both primers have 3' T, and are abutted to the CpG, forcing a TaqI restriction site if the CpG is intact. Typically, a 52 bp PCR product is then cut in half. CpG-PCR also illustrates that PAGE-MADGE readily permits analysis of 'ultrashort' PCRs. Melt-MADGE employs real-time-variable-temperature electrophoresis to examine duplex mobility during melting, achieving DGGE-like de novo mutation scanning, but with the conveniences of arbitrary programmability, MADGE compatibility and short run time. This suite of methods enhances our capability to type or scan thousands of samples simultaneously, by 10-100-fold.


The Escherichia coli DNA mismatch repair protein, MutS, binds single base pair mismatches and short deletions in vivo and in vitro. To adapt this protein for mutation detection, a fusion protein of E. coli MutS with a biotinylated peptide domain has been constructed (MutSb). The biotinylation tag facilitates MutS detection and binding by avidin without significantly altering the DNA mismatch binding properties of MutS in vitro. We describe a novel and rapid mutation detection
method with MutSb using streptavidin-coated magnetic beads and demonstrate that MutSb can also be used to remove mismatch containing DNA fragments from a mixture of DNA fragments in solution.


http://www.sciencedirect.com/science/article/B6T72-4840KXF-Y/2/9c8972b5c782f1d500c5c5dd1bda9aba

We have developed a method for the whole sequence amplification of yeast artificial chromosome (YAC) DNA excised from preparative pulsed-field gel electrophoresis using single unique primer-polymerase chain reaction procedures. We used seven contiguous YAC clones, which span 2 Mbp of the Huntington disease gene region on 4p16.3, to amplify the YAC DNAs. The average size of the amplified DNA was ~300 bp long, and 12 DNA markers located on the YAC clones positively hybridized with these amplified products, implying that the sequences of the YAC clones were comprehensively amplified by our procedures. These amplified YAC DNAs greatly facilitate the characterization of YAC clones, leading to the detailed analysis of the defined chromosomal region.


http://www.sciencedirect.com/science/article/B6T72-497C71D-7/2/8ec5523c376be722f9a414b9041d361a

Degenerate oligonucleotide primers were made to peptide sequences from hydroxylamine oxidoreductase (HAO) from Nitrosomonas europaea. The primers were used singly in PCR reactions to amplify portions of the gene for HAO from genomic DNA. Southern hybridizations using fragments amplified with each primer showed that they labeled the same genomic DNA fragments. The PCR-amplified fragments were successfully used to screen a gene library for clones containing the HAO gene. The method of isolating genes by PCR with single primers has general utility.


http://www.sciencedirect.com/science/article/B6T72-3XX6S3X-M/2/03c6a4faa66fefd7cc35d143787f1d97

The SuperTth DNA polymerase from Thermus thermophilus exhibits template-independent terminal transferase (extendase) activity. This enzyme is proposed as a cheap alternative for both high performance PCR as well as quick T-vector cloning of amplicons, including reverse transcription and cDNA cloning.

http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-8/2/bcfa9c411e26053f1f54927dc2961c18

Large-scale screening for known polymorphisms will require techniques with few steps and the ability to automate each of these steps. In this regard, the 5' nuclease, or TaqMan, PCR assay is especially attractive. A fluorogenic probe, consisting of an oligonucleotide labeled with both a fluorescent reporter dye and a quencher dye, is included in a typical PCR. Amplification of the probe-specific product causes cleavage of the probe, generating an increase in reporter fluorescence. By using different reporter dyes, cleavage of allele-specific probes can be detected in a single PCR. The 5' nuclease assay has been successfully used to discriminate alleles that differ by a single base substitution. Guidelines have been developed so that an assay for any single nucleotide polymorphism (SNP) can be quickly designed and implemented. All assays are performed using a single reaction buffer and single thermocycling protocol. Furthermore, a standard method of analysis has been developed that enables automated genotype determination. Applications of this assay have included typing a number of polymorphisms in human drug metabolism genes.


http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-9/2/7726ec68e808881a0c465159bbd67a26

We demonstrate that single-nucleotide differences in a DNA sequence can be detected in homogeneous assays using molecular beacons. In this method, the region surrounding the site of a sequence variation is amplified in a polymerase chain reaction and the identity of the variant nucleotide is determined by observing which of four differently colored molecular beacons binds to the amplification product. Each of the molecular beacons is perfectly complementary to one variant of the target sequence and each is labeled with a different fluorophore. To demonstrate the specificity of these assays, we prepared four template DNAs that only differed from one another by the identity of the nucleotide at one position. Four amplification reactions were prepared, each containing all four molecular beacons, but each initiated with only one of the four template DNAs. The results show that in each reaction a fluorogenic response was elicited from the molecular beacon that was perfectly complementary to the amplified DNA, but not from the three molecular beacons whose probe sequence mismatched the target sequence. The color of the fluorescence that appeared in each tube during the course of the amplification indicated which nucleotide was present at the site of variation. These results demonstrate the extraordinary specificity of molecular beacons. Furthermore, the results illustrate how the ability to label molecular beacons with differently colored fluorophores enables simple multiplex assays to be carried out for genetic analysis.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2P/2/e12ab1d091466044db5a8c03c0132195
We have used analysis of ethidium-bromide-stained reverse transcriptase-polymerase chain reaction (RT-PCR) products to assess the effects of X-chromosome inactivation during spermatogenesis in the mouse. RT-PCR was performed on total RNA from eight different spermatogenic cell types, including premeiotic spermatogonia, meiotic spermatocytes, and postmeiotic spermatids, to detect transcripts from five different X-linked structural genes (Pgk-1, Zfx, Pdha-1, Hprt, and Phka) and two autosomal genes (Pgk-2 and [beta]-actin). Relative intensities of ethidium-bromide-strained RT-PCR products representing transcripts from each gene in each cell type were analyzed by densitometry using the Image program (version 1.4, NIH), and normalized against [beta]-actin values. These results suggest a coordinate inactivation of the X-linked loci at the onset of meiosis, followed by variable rates of decline of corresponding transcript levels reflecting differential mRNA stabilities and/or leaky expression after inactivation. Technically, these results indicate that analysis of ethidium-bromide-stained RT-PCR products can be used to provide a "semiquantitative" indication of relative levels of specific transcripts in a developing cell lineage without using radioactive probes to quantitate these products.


http://www.sciencedirect.com/science/article/B6T72-476VPM2-52/2/bc8c994e918d0f51a73c6db6812225d61


http://www.sciencedirect.com/science/article/B6T72-4840M30-2V/2/60157c05cb23a511a4026cd9faa3f80f

A new and potentially reliable method for the isolation of yeast artificial chromosome (YAC)-insert termini, termed junction trapping, is described. This method is based on simple partial digestions of the YAC-containing yeast strain, ligation to a plasmid vector, and selection of the termini by two rounds of polymerase chain reaction (PCR). To date, the method has generated both terminal sequences from each of nine YACs (100%) that have been examined.


http://www.sciencedirect.com/science/article/B6T72-4840KVHB/2/56bdccf373c80e48e9217e5d0eb5a8f

The potential usefulness of chromosome microdissection, the polymerase chain reaction (PCR), and dot blot hybridization as a quick screening method for determining the genetic composition of double minute chromosomes (DMs) was evaluated. DMs or abnormally banding regions (ABRs) were microdissected from from multidrug-resistant hamster cell lines and amplified with PCR using primers specific for the hamster multidrug-resistance (MDR) gene, pgp 1. The microdissected-PCR-amplified products were shown to (a) hybridize to a 32P-labeled pCHP1 probe for the hamster MDR gene by using dot blot or Southern blot analysis and also (b) hybridize back to the chromosome region from which they were originally dissected by using fluorescent in situ hybridization. Microdissected/PCR-amplified DMs were also shown to hybridize
to ABRs. When microdissected DMs and ABRs were amplified using hamster specific Alu primers, the resulting material was shown to hybridize with probes for hamster MDR and Alu. These results suggest that the DMs contained in these MDR hamster cell lines contain Alu-like sequences and the chromosome microdissection-PCR-hybridization approach might be used as a quick screening method for identifying genes amplified in DMs and ABRs in cell lines and human tumor samples.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2M/2/ee21f9d29b702e9c96c572cb7e7e9815

This article describes a method for determining whether a particular nucleic acid sequence is present in a sample and for discriminating between any two nucleic acid sequences if such sequences differ only by a single nucleotide. The method entails extension of a novel two-component primer on templates that may or may not include a target nucleic acid sequence. The 3' portion of the primer is complementary to a portion of the template adjacent to the target sequence (for example, the polymorphic nucleotide). The 5' portion of the primer is complementary to a different preselected nucleic acid sequence. Extension of the 3' portion of the primer with a labeled deoxynucleoside triphosphate yields a labeled extension product, but only if the template includes the target sequence. The presence of such a labeled primer-extension product is detected by hybridization of the 5' portion to the preselected sequence. The preselected sequence is immobilized on a solid support. The method has been applied to genotyping individuals for the two-allele polymorphism of the human tyrosinase gene.


Direct label alkaline phosphatase (AP) conjugated oligonucleotide probes (AP-DNA) were prepared to assess their utility for allele-specific detection of single base substitutions. Oligonucleotide conjugates were designed to detect point mutations in the genes for lipoprotein lipase (LPL) and coagulation factor-V (FV). Genomic DNA samples, including ones known to harbor point mutations in the genes for LPL and FV, were prepared from whole blood and subjected to polymerase chain reaction (PCR). PCR products were analyzed by Southern hybridization with the allele-specific AP-DNA probes and restriction endonuclease analysis. Thermal profiles for hybridization indicate optimal allele-specific selectivity was achieved with temperatures ranging from 45[deg]C to 55[deg]C at a total Na+ concentration of 150 mM. Under these conditions the base changes studied were easily discriminated with allele specific hybridization signals in excess of 200:1 as estimated by scanning densitometry. Complete concordance was observed between hybridization and restriction analyses for 175 LPL and 201 FV clinical and reference samples. The total time for analysis of the PCR products was less than 2 h with a dot blot hybridization protocol.

http://www.sciencedirect.com/science/article/B6T2D-45KT0KY-3/2/8c513c577d359ce72d07305926844f43

The C57BL/6J-Min/+ (multiple intestinal neoplasia) mouse has a heterozygous nonsense ApcMin (adenomatous polyposis coli) mutation, and numerous adenomas spontaneously develop in the intestine. Neonatal exposure of Min/+ mice to the food carcinogens 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (one injection of 50 mg/kg) increased the number of small intestinal tumours about three- and two-fold, respectively. The number of colonic tumours was only increased in males. We examined whether the wild-type Apc allele was affected in intestinal tumours induced by either PhIP or IQ. In spontaneously formed and in IQ-induced small intestinal and colonic tumours from these mice, the main mechanism for tumour induction was loss of wild-type Apc allele, i.e. loss of heterozygosity (LOH). In contrast to the IQ-induced (84% LOH) and spontaneously (88% LOH) formed tumours, only 55% of the PhIP-induced small intestinal tumours from males showed LOH. Tumours that apparently had retained the wild-type Apc allele were further analysed for the presence of truncated Apc proteins by the in vitro synthesised protein (IVSP) assay. Truncated Apc proteins, indicating truncation mutations in exon 15 of the Apc gene, were detected in two of the 12 PhIP-induced tumours in segment 2 (codons 686-1217), and two of five IQ-induced tumours, one in segment 2 and the other in segment 3 (codons 1099-1693). Three of these four mutations, all in segment 2 of the Apc gene, were confirmed by sequencing. The PhIP-induced mutations were detected at codon 1125 (C deletion) and 1130 (G-T transversion), and the IQ-induced mutation was at codon 956 (C-T transition). Importantly, no truncated proteins were detected in tumours from unexposed mice with apparently retained wild-type Apc allele. These results show that one injection of either PhIP or IQ induces intestinal tumours in the Min/+ mice by inactivation of the wild-type Apc allele either by causing LOH or truncation mutations.


http://www.sciencedirect.com/science/article/B6T2D-3XNVJ81-8/2/2d3846166dcb3891bb56e76c9d1be2db

Transgenic mice containing multiple copies of the [Phi]X174 am3 allele are being developed as a model for detecting tissue-specific in vivo mutation. In order to derive an analogous system for measuring am3 mutation in vitro, cells were cultured from 15-day-old C57Bl/6J mouse embryos that were homozygous for the transgene and these cells were transfected with a plasmid expressing the SV40 large T-antigen. Two G418-resistant colonies were isolated from this culture and expanded to continuously proliferating cell lines (PX-1 and PX-2). Line PX-2 was treated with up to 1.0 mg/ml of N-ethyl-N-nitrosourea (ENU), assayed for survival by cloning efficiency after overnight culture, and assayed for am3 mutations after 5 days of culture. Survival decreased to 31% at the highest dose of ENU, while mutant frequency increased with dose from approximately

We performed a study on Belarusian "liquidators", exploring whether increase in the frequencies of germline mutations at microsatellite loci could be found in their progeny. The liquidators, mostly young males, were those involved (during 1986 and 1987) in clean-up operations in the radioactively contaminated area following the Chernobyl nuclear power plant accident in 1986. Many liquidators fathered children during the clean-up period and after the work had been terminated. The numbers of families studied were 64 (liquidators) and 66 (controls). A total of 72 loci (31 autosomal, one X-linked and 40 Y-linked) were used. DNA was isolated from peripheral blood lymphocytes and the microsatellite loci were amplified by the polymerase chain reaction with fluorescence-labelled primers. Mutations were detected as variations in the length of the loci. At the Y-linked loci, the mutation rates (expressed as number of mutations among the total number of loci for the individuals included) are $2.9 \times 10^{-3}$ (4/1392) and $2.1 \times 10^{-3}$ (3/1458) in the children of exposed and control parents, respectively. This difference is not statistically significant. At the autosomal loci, the corresponding estimates are $5.9 \times 10^{-3}$ (11/1862; exposed group) and $8.5 \times 10^{-3}$ (18/2108; control). Again, the difference is not significant. The possibility that the Belarusian population might have been unexpectedly exposed to some chemical contaminants in the environment appears unlikely in view of the finding that the spontaneous mutation rates at the same set of loci in several non-Belarusian populations were similar to those in Belarus. The estimated mean radiation dose to the liquidators was small, being about 39 mSv, and this might be one reason why no increases in mutation rates due to radiation could be found.


Etoposide is among the most widely used anti-cancer drugs. Its use, however, has been associated with increased risk of secondary acute myeloid leukemia (AML) which is characterized by chromosomal translocations suggesting involvement of recombination-associated motifs at the breakpoints. A PCR-based assay was developed to quantitate the frequency of two illegitimate V(D)J recombinase-mediated genomic rearrangements—a 20-kb deletion in the hprt gene and the $t(14;18)$ translocation found in non-Hodgkin's lymphoma. We examined both lymphocyte and non-lymphocyte blood cell DNA of children with acute lymphoblastic leukemia (ALL) for changes in the frequencies of these biomarkers during etoposide therapy to determine the level of illegitimate V(D)J recombination changes during therapy. A low level of $t(14;18)$ was found in the lymphocytes before etoposide treatment, which was significantly reduced during etoposide therapy. In before-etoposide samples, no $t(14;18)$ were found among $7.72 \times 10^{7}$ non-
lymphocytes; during treatment none were found among 1.87 x 10^8 non-lymphocytes. Deletions were not found before etoposide treatment in either the lymphocytes (6.67 x 10^7) or non-lymphocytes (5.43 x 10^7) and were non-significantly elevated during etoposide therapy (1 in 1.4 x 10^8 lymphocytes and 1 in 1.39 x 10^8 non-lymphocytes). It is interesting to note the one patient with an hprt deletion mutation in non-lymphocytes; V(D)J recombination is not normally found in this cell type, but is the cell type from which AML derives. Several patients had clones of t(14;18)-bearing cells as determined by DNA sequence analysis. These results suggest that this etoposide-based chemotherapy was ineffective in producing genomic rearrangements mediated by illegitimate V(D)J recombination in these patients.


http://www.sciencedirect.com/science/article/B6T2D-48BM66N-1/2/c5423a539d9817f72bd37549e8e5e

Potassium bromate (KBrO3) induces DNA damage and tumors in mice and rats, but is a relatively weak mutagen in microbial assays and the in vitro mammalian Hprt assay. Concern that there may be a human health risk associated with bromate, a disinfectant by-product of ozonation, has accompanied the increasing use of ozonation as an alternative to chlorination for treatment of drinking water. In this study, we have evaluated the mutagenicity of KBrO3 and sodium bromate (NaBrO3) in the Tk gene of mouse lymphoma cells. In contrast to the weak mutagenic activity seen in the previous studies, bromate induced a mutant frequency of over 100 x 10^-6 at 0.6 mM with minimal cytotoxicity (70-80% survival) and over 1300 x 10^-6 at 3 mM (~10% survival). The increase in the Tk mutant frequency was primarily due to the induction of small colony of Tk mutants. Loss of heterozygosity (LOH) analysis of 384 mutants from control and 2.7 mM KBrO3-treated cells showed that almost all (99%) bromate-induced mutants resulted from LOH, whereas in the control cultures 77% of the Tk mutants were LOH. Our results suggest that bromate is a potent mutagen in the Tk gene of mouse lymphoma cells, and the mechanism of action primarily involves LOH. The ability of the mouse lymphoma assay to detect a wider array of mutational events than the microbial or V79 Hprt assays may account for the potent mutagenic response.


http://www.sciencedirect.com/science/article/B6T2D-45NGSX6-1/2/72c28f66445465d05bc80de191c6bac6

Allele-specific competitive blocker PCR (ACB-PCR) is a sensitive allele-specific amplification method in which preferential amplification of the mutant allele occurs by using a primer that has more mismatches to the wild-type allele than to the mutant allele (mutant-specific primer, MSP). Additionally, a non-extendable primer with more mismatches to the mutant allele than to the wild-type allele (blocker primer, BP) competes with the MSP for binding to the wild-type allele, thereby reducing background amplification from the wild-type allele. ACB-PCR primer design is largely dependent upon the basepair substitution being measured, making it unclear if this method is broadly applicable. In an earlier study, an H-ras codon 61 CAA->AAA mutation had been detected by ACB-PCR at a sensitivity of 10-5. In this study, ACB-PCR was applied to two human K-ras codon 12 mutations: GGT->GTT and GGT->GAT. The method was optimized by systematically altering the concentrations of Perfect Match PCR Enhancer, MSP, BP, and dNTPs. For each mutation, mutant fractions as low as 10-5 were detected, indicating that this assay can
be used on a variety of base substitution mutations. In addition, the results suggest that the 3’-terminal mismatches between the MSP and wild-type allele may be used to predict the ACB-PCR conditions that will be appropriate for the detection of other base substitution mutations. The range of concentrations for each of these components is narrow, making this method relatively easy to apply to additional mutational targets.


http://www.sciencedirect.com/science/article/B6T2D-4B7YPB6-1/2/4dbb27ff0716869ef6f5221ff1f4c8b3f

The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induces intestinal tumours in C57BL/6J-multiple intestinal neoplasia (Min)/+ mice. The main mechanism for PhIP-induced tumour induction in Min/+ mice is loss of the wild-type adenomatous polyposis coli (Apc) allele, i.e. loss of heterozygosity (LOH). In this study, single injections of either 10, 17.5 or 25 mg/kg PhIP on days 3-6 after birth all increased the mean number of small intestinal tumours two to three-fold, from 37.7 in controls to 124.8 in the PhIP-treated Min/+ mice. In total, we analysed 292 small intestinal tumours and 253 of these had LOH. The frequency of LOH in the Apc gene was 88, 93, 83 and 84% in tumours of 0, 10, 17.5 and 25 mg/kg PhIP-treated mice, respectively. Therefore, these lower doses of PhIP did not reduce the frequency of LOH, as found in our previous study with a single injection of 50 mg/kg PhIP (Mutat. Res. 1-2 (2002) 157). In the second part of this study, we wanted to characterise Apc truncation mutations from tumour samples apparently retaining the Apc wild-type allele from this and two previous experiments with PhIP-exposed Min/+ mice. In the first half of exon 15 in Apc, we verified 25 mutations from 804 tumour samples of PhIP-treated mice. Of these were 60% G->T transversions, and 16% G deletions, indicating that these are the predominant types of PhIP-induced truncation mutations in the Apc gene in Min/+ mice. Most of the mutations were located between codon 989 and 1156 corresponding to the first part of the [beta]-catenin binding region. We also identified two Apc truncation mutations from 606 spontaneously formed intestinal tumours from untreated Min/+ mice, one C->T transition and one T insertion, which were different from those induced by PhIP.


http://www.sciencedirect.com/science/article/B6T2D-3XMGP4W-D/2/cbfa31dae84ebe5df75801eb78f059b1

This paper describes the spectrum of mutations induced by 4-nitroquinoline N-oxide (4-NQO) in the lacI target gene of the transgenic Big Blue(R) Rat2 cell line. There are only a few report for the mutational spectrum of 4-NQO in a mammalian system although its biological and genetic effects have been well studied. Big Blue(R) Rat2 cells were treated with 0.03125, 0.0625 or 0.125 [mu]g/ml of 4-NQO, the highest concentration giving 85% survival. Our results indicated that the mutant frequency (MF) induced by 4-NQO was dose-dependent with increases from three- to seven-fold. The DNA sequence analysis of lacI mutants from the control and 4-NQO treatment groups revealed an obvious difference in the spectra of mutations. In spontaneous mutants, transition (60%) mutations, especially G:C->A:T transition (45%), were most frequent. However, the major type of base substitution after treatment of 4-NQO was transversions (68.8%), especially G:C->T:A (43.8%), while only 25% of mutants were transitions. These results are
consistent with those produced by 4-NQO in other systems and the transgenic assay system will be a powerful tool to postulate more accurately the mechanism of chemical carcinogenesis involved.


http://www.sciencedirect.com/science/article/B6T2D-45JYJTV-2/2/b66717cb9b7e22b72f08dbff71080293

The aim of this study was to use DNA adducts levels, detected by 32P-postlabelling, as a biomarker to assess human exposure to polycyclic aromatic hydrocarbons (PAHs) from a coke oven plant and explore the possible association between CYP1A1 MspI, GSTP1, GSTM1 and GSTT1 genotypes, and smoking status on bulky DNA adduct formation. A large amount of inter-individual variation in adduct level was observed among workers with the same job and the same smoking habits. No significant differences were observed in DNA adduct levels between the coke oven workers and control group. Smokers in the control group had significantly higher DNA adducts than the non-smokers in the same group (35.13+/−21.11 versus 11.18+/−8.00, per 108 nucleotides, P=0.003). In this group, the correlation between the level of DNA adducts and the cigarettes smoked was strongly significant (r=0.70, P=0.003). These results suggests that tobacco smoke could behave as a confounding factor for evaluation of DNA adducts arising from occupational exposure. The levels of DNA adducts in smokers not occupationally exposed to PAHs is dependent on the polymorphisms CYP1A1 MspI in the 3’ non-coding region (49.04+/−22.18 versus 25.85+/−15.87, per 108 nucleotides, PGST genotypes studied.


http://www.sciencedirect.com/science/article/B6T2D-4BG45X0-1/2/f0ed3b1cc57d8ec4a2aa3fb870da4b5e

1,3-Butadiene (BD) is an important industrial chemical and pollutant. Its ability to induce genetic damage and cause hematological malignancies in humans is controversial. We have examined chromosome damage by fluorescence in situ hybridization (FISH) and mutations in the HPRT gene in the blood of Chinese workers exposed to BD. Peripheral blood samples were collected and cultured from 39 workers exposed to BD (median level 2 ppm, 6 h time-weighted average) and 38 matched controls in Yanshan, China. No difference in the level of aneuploidy or structural changes in chromosomes 1, 7, 8, and 12 was detected in metaphase cells from exposed subjects in comparison with matched controls, nor was there an increase in the frequency of HPRT mutations in the BD-exposed workers. Because genetic polymorphisms in glutathione S-transferase (GST) enzymes and microsomal epoxide hydrolase (EPHX1) may affect the genotoxic effects of BD and its metabolites, we also related chromosome alterations and gene mutations to GSTT1, GSTM1 and EPHX1 genotypes. Overall, there was no effect of variants in these genotypes on numerical or structural changes in chromosomes 1, 7, 8 and 12 or on HPRT mutant frequency in relation to BD exposure, but the GST genotypes did influence background levels of both hyperdiploidy and HPRT mutant frequency. In conclusion, our data show no increase in chromosomal aberrations or HPRT mutations among workers exposed to BD, even in potentially susceptible genetic subgroups. The study is, however, quite small and the levels of BD exposure are not extremely high, but our findings in China do support those from a similar study
conducted in the Czech Republic. Together, these studies suggest that low levels of occupational BD exposure do not pose a significant risk of genetic damage.


http://www.sciencedirect.com/science/article/B6T2D-45H93HP-1/2/ac9e1b1989d6a6d159aa45fe0791717b

A method to measure the germline mutations induced by cancer treatment in humans is needed. To establish such a method we used a transgenic mouse model consisting of a human DNA repeat locus that has a high spontaneous mutation frequency as a biomarker. Alterations in repeat number were measured in individual sperm from mice hemizygous for an expanded (CTG)162 human myotonic dystrophy type 1 (DM1) microsatellite repeat using single genome-equivalent (g.e.) PCR and detection by a DNA fragment analyzer. Mutation frequencies were measured in DNA from sperm from controls and sperm derived from stem spermatogonia, differentiating spermatogonia, and spermatocytes exposed to radiation and from spermatocytes of mice treated with cyclophosphamide. There was no increase above control levels in mutations, scored as >1 repeat changes, in any of the treated groups. However, moderately large deletion mutants (between 9 and 20 repeat changes) were observed at frequencies of 2.2% when spermatocytes were treated with cyclophosphamide and, 1.8 and 2.5% when spermatocytes and stem cells, respectively, were treated with radiation, which were significantly higher than the frequency of 0.3% in controls. Thus, radiation and cyclophosphamide induced deletions in the expanded DM1 trinucleotide repeat. PCR artifacts were characterized in sperm DNA from controls and from mice treated with radiation; all artifacts involved losses of more than 20 DM1 repeats, and surprisingly the artifact frequency was higher in treated sperm than in control sperm. The radiation-induced increase in the frequency of PCR artifacts might reflect alterations in sperm DNA that destabilize the genome not only during PCR amplification but also during early embryonic development.

Genetics (30)


http://www.genetics.org/cgi/content/abstract/161/2/813

Determination of parentage is fundamental to the study of biology and to applications such as the identification of pedigrees. Limitations to studies of parentage have stemmed from the use of an insufficient number of hypervariable loci and mismatches of alleles that can be caused by mutation or by laboratory error and that can generate false exclusions. Furthermore, most studies of parentage have been limited to comparisons of small numbers of specific parent-progeny triplets thereby precluding large-scale surveys of candidates where there may be no prior knowledge of parentage. We present an algorithm that can determine probability of parentage in circumstances where there is no prior knowledge of pedigree and that is robust in the face of missing data or mistyped data. We present data from 54 maize hybrids and 586 maize inbreds.
that were profiled using 195 SSR loci including simulations of additional levels of missing and mistyped data to demonstrate the utility and flexibility of this algorithm.


http://www.genetics.org/cgi/content/abstract/169/2/1009

Plant endo-(β)-1,3-glucanases (EGases) degrade the cell wall polysaccharides of attacking pathogens and release elicitors of additional plant defenses. Isozymes EGaseA and EGaseB of soybean differ in susceptibility to a glucanase inhibitor protein (GIP1) produced by Phytophthora sojae, a major soybean pathogen. EGaseA, the major elicitor-releasing isozyme, is a high-affinity ligand for GIP1, which completely inhibits it, whereas EGaseB is unaffected by GIP1. We tested for departures from neutral evolution on the basis of partial sequences of EGaseA and EGaseB from 20 widespread accessions of Glycine soja (the wild progenitor of soybean), from 4 other Glycine species, and across dicotyledonous plants. G. soja exhibited little intraspecific variation at either locus. Phylogeny-based codon evolution models detected strong evidence of positive selection on Glycine EGaseA and weaker evidence for selection on dicot EGases and Glycine EGaseB. Positively selected peptide sites were identified and located on a structural model of EGase bound to GIP1. Positively selected sites and highly variable sites were found disproportionately within 4.5 A of bound GIP1. Low variation within G. soja EGases, coupled with positive selection in both Glycine and dicot lineages and the proximity of rapidly evolving sites to GIP1, suggests an arms race involving repeated adaptation to pathogen attack and inhibition.


http://www.genetics.org/cgi/content/abstract/167/2/941

Sequencing was used to investigate the origin of the D genome of the allopolyploid species Triticum aestivum and Aegilops cylindrica. A 247-bp region of the wheat D-genome Xwye838 locus, encoding ADP-glucopyrophosphorylase, and a 326-bp region of the wheat D-genome Gss locus, encoding granule-bound starch synthase, were sequenced in a total 564 lines of hexaploid wheat (T. aestivum, genome AABBDD) involving all its subspecies and 203 lines of Aegilops tauschii, the diploid source of the wheat D genome. In Ae. tauschii, two SNP variants were detected at the Xwye838 locus and 11 haplotypes at the Gss locus. Two haplotypes with contrasting frequencies were found at each locus in wheat. Both wheat Xwye838 variants, but only one of the Gss haplotypes seen in wheat, were found among the Ae. tauschii lines. The other wheat Gss haplotype was not found in either Ae. tauschii or 70 lines of tetraploid Ae. cylindrica (genomes CCDD), which is known to hybridize with wheat. It is concluded that both T. aestivum and Ae. cylindrica originated recurrently, with at least two genetically distinct progenitors contributing to the formation of the D genome in both species.


http://www.genetics.org/cgi/content/abstract/162/1/307
ROSA22 male mice are sterile due to a recessive gene-trap mutation that affects development of the spermatid flagellum. The defect involves the flagellar axoneme, which becomes unstable around the time of its assembly. Despite a subsequent complete failure in flagellar assembly, development of the spermatid head appears normal and the spermatid head is released at the correct stage in spermatogenesis. The mutation is pleiotropic. Although ROSA22 homozygote males have normal levels of circulating testosterone and display normal mating behavior, they do not exhibit intermale aggressive behavior and have reduced body fat. The mutated gene (Gtrgeo22) maps to mouse chromosome 10 and is closely flanked by two known genes, Madcam1 and Cdc34. Ribonuclease protection analysis indicates that expression of the flanking genes is unaffected by the mutation. Gtrgeo22 is expressed at low levels in epithelial cells in several tissues, as well as in testis and brain. Analysis of the peptide coding sequence suggests that Gtrgeo22 encodes a novel transmembrane protein, which contains dileucine and tyrosine-based motifs involved in intracellular sorting of transmembrane proteins. Analysis of the Gtrgeo22 gene product should provide novel insight into the molecular basis for intermale aggression and sperm flagellar development.


http://www.genetics.org/cgi/content/abstract/165/2/601

In a screen for suppressors of the Drosophila winglessPE4 nonsense allele, we isolated mutations in the two components that form eukaryotic release factor. eRF1 and eRF3 comprise the translation termination complex that recognizes stop codons and catalyzes the release of nascent polypeptide chains from ribosomes. Mutations disrupting the Drosophila eRF1 and eRF3 show a strong maternal-effect nonsense suppression due to readthrough of stop codons and are zygotically lethal during larval stages. We tested nonsense mutations in wg and in other embryonically acting genes and found that different stop codons can be suppressed but only a subset of nonsense alleles are subject to suppression. We suspect that the context of the stop codon is significant: nonsense alleles sensitive to suppression by eRF1 and eRF3 encode stop codons that are immediately followed by a cytidine. Such suppressible alleles appear to be intrinsically weak, with a low level of readthrough that is enhanced when translation termination is disrupted. Thus the eRF1 and eRF3 mutations provide a tool for identifying nonsense alleles that are leaky. Our findings have important implications for assigning null mutant phenotypes and for selecting appropriate alleles to use in suppressor screens.


http://www.genetics.org/cgi/content/abstract/167/4/1663

The impact of ploidy on adaptation is a central issue in evolutionary biology. While many eukaryotic organisms exist as diploids, with two sets of gametic genomes residing in the same nucleus, most basidiomycete fungi exist as dikaryons in which the two genomes exist in separate nuclei that are physically paired and that divide in a coordinated manner during hyphal extension. To determine if haploid monokaryotic and dikaryotic mycelia adapt to novel environments under natural selection, we serially transferred replicate populations of each ploidy state on minimal medium for 18 months (≈13,000 generations). Dikaryotic mycelia responded to selection with increases in growth rate, while haploid monokaryotic mycelia did not. To determine if the haploid components of the dikaryon adapt reciprocally to one another's presence over time, we recovered the intact haploid components of dikaryotic mycelia at different time points (without meiosis) and mated them with nuclei of different evolutionary histories. We found evidence for coadaptation
between nuclei in one dikaryotic line, in which a dominant deleterious mutation in one nucleus was followed by a compensatory mutation in the other nucleus; the mutant nuclei that evolved together had the best overall fitness. In other lines, nuclei had equal or higher fitness when paired with nuclei of other histories, indicating a heterozygote advantage. To determine if genetic exchange occurs between the two nuclei of a dikaryon, we developed a 24-locus genotyping system based on single nucleotide polymorphisms to monitor somatic exchange. We observed genetic exchange and recombination between the nuclei of several different dikaryons, resulting in genotypic variation in these mitotic cell lineages.


http://www.genetics.org/cgi/content/abstract/genetics.104.036400v1

The involucrin gene encodes a protein of terminally differentiated keratinocytes. Its segment of repeats, which represents up to 80% of the coding region, is highly polymorphic in mouse strains derived from wild progenitors. Polymorphism includes nucleotide substitutions, but is most strikingly due to the recent addition of variable number of repeats at a precise location within the segment of repeats. Each mouse taxon examined showed consistent and distinctive patterns of evolution of its variable region: very rapid changes in most M. m. domesticus alleles, slow changes in M. m. musculus and complete arrest in M. spretus. We conclude that changes in the variable region are controlled by the genetic background. One of the M. m. domesticus alleles (DIK-L), which is of M. m. musculus origin, has undergone a recent repeat duplication typical of M. m. domesticus. This suggests that the genetic background controls repeat duplications through trans-acting factors. Because the repeat pattern differs in closely related murine taxa, involucrin reveals with greater sensitivity than random nucleotide substitutions the evolutionary relations of the mouse and probably of all murids.


http://www.genetics.org/cgi/content/abstract/161/4/1561

Of the seven recognized species of the Anopheles gambiae complex, A. gambiae s.s. is the most widespread and most important vector of malaria. It is becoming clear that, in parts of West Africa, this nominal species is not a single panmictic unit. We found that the internal transcribed spacer (ITS) of the X-linked rDNA has two distinct sequences with three fixed nucleotide differences; we detected no heterozygotes at these three sites, even in areas of sympatry of the two ITS types. The intergenic spacer (IGS) of this region also displays two distinct sequences that are in almost complete linkage disequilibrium with the distinct ITS alleles. We have designated these two types as S/type I and M/type II. These rDNA types correspond at least partly to the previously recognized chromosomal forms. Here we expand the geographic range of sampling to 251 individuals from 38 populations. Outside of West Africa, a single rDNA type, S/type I, corresponds to the Savanna chromosomal form. In West Africa, both types are often found in a single local sample. To understand if these findings might be due to unusual behavior of the rDNA region, we sequenced the same region for 46 A. arabiensis, a sympatric sibling species. No such distinct discontinuity was observed for this species. Autosomal inversions in one chromosome arm (2R), an insecticide resistance gene on 2L, and this single X-linked region indicate at least two genetically differentiated subpopulations of A. gambiae. Yet, rather extensive studies of other regions of the genome have failed to reveal genetic discontinuity. Evidently, incomplete genetic isolation exists within this single nominal species.

http://www.genetics.org/cgi/content/abstract/162/4/1979

The clustering of telomeres on the nuclear envelope (NE) during meiotic prophase to form the bouquet arrangement of chromosomes may facilitate homologous chromosome synapsis. The pam1 (plural abnormalities of meiosis 1) gene is the first maize gene that appears to be required for telomere clustering, and homologous synapsis is impaired in pam1. Telomere clustering on the NE is arrested or delayed at an intermediate stage in pam1. Telomeres associate with the NE during the leptotene-zygotene transition but cluster slowly if at all as meiosis proceeds. Intermediate stages in telomere clustering including miniclusters are observed in pam1 but not in wild-type meiocytes. The tight bouquet normally seen at zygotene is a rare event. In contrast, the polarization of centromeres vs. telomeres in the nucleus at the leptotene-zygotene transition is the same in mutant and wild-type cells. Defects in homologous chromosome synapsis include incomplete synapsis, nonhomologous synapsis, and unresolved interlocks. However, the number of RAD51 foci on chromosomes in pam1 is similar to that of wild type. We suggest that the defects in homologous synapsis and the retardation of prophase I arise from the irregularity of telomere clustering and propose that pam1 is involved in the control of bouquet formation and downstream meiotic prophase I events.


http://www.genetics.org/cgi/content/abstract/167/1/367

The human X chromosome exhibits four "evolutionary strata," interpreted to represent distinct steps in the process whereby recombination became arrested between the proto X and proto Y. To test if this is a general feature of sex chromosome evolution, we studied the Z-W sex chromosomes of birds, which have female rather than male heterogamety and evolved from a different autosome pair than the mammalian X and Y. Here we analyze all five known gametologous Z-W gene pairs to investigate the "strata" hypothesis in birds. Comparisons of the rates of synonymous substitution and intronic divergence between Z and W gametologs reveal the presence of at least two evolutionary strata spread over the p and q arms of the chicken Z chromosome. A phylogenetic analysis of intronic sequence data from different avian lineages indicates that Z-W recombination ceased in the oldest stratum (on Zq; CHD1Z, HINTZ, and SPINZ) 102-170 million years ago (MYA), before the split of the Neoaves and Eoaves. However, recombination continued in the second stratum (on Zp; UBAP2Z and ATP5A1Z) until after the divergence of extant avian orders, with Z and W diverging 58-85 MYA. Our data suggest that progressive and stepwise cessation of recombination is a general feature behind sex chromosome evolution.


http://www.genetics.org/cgi/content/abstract/161/1/231

The neural selector gene cut, a homeobox transcription factor, is required for the specification of
the correct identity of external (bristle-type) sensory organs in Drosophila. Targets of cut function, however, have not been described. Here, we study bereft (bft) mutants, which exhibit loss or malformation of a majority of the interommatidial bristles of the eye and cause defects in other external sensory organs. These mutants were generated by excising a P element located at chromosomal location 33AB, the enhancer trap line E8-2-46, indicating that a gene near the insertion site is responsible for this phenotype. Similar to the transcripts of the gene nearest to the insertion, reporter gene expression of E8-2-46 coincides with Cut in the support cells of external sensory organs, which secrete the bristle shaft and socket. Although bft transcripts do not obviously code for a protein product, its expression is abolished in bft deletion mutants, and the integrity of the bft locus is required for (interommatidial) bristle morphogenesis. This suggests that disruption of the bft gene is the cause of the observed bristle phenotype. We also sought to determine what factors regulate the expression of bft and the enhancer trap line. The correct specification of individual external sensory organ cells involves not only cut, but also the lineage genes numb and tramtrack. We demonstrate that mutations of these three genes affect the expression levels at the bft locus. Furthermore, cut overexpression is sufficient to induce ectopic bft expression in the PNS and in nonneuronal epidermis. On the basis of these results, we propose that bft acts downstream of cut and tramtrack to implement correct bristle morphogenesis.


http://www.genetics.org/cgi/content/abstract/genetics.104.039735v1

The Wingless(Wg)/Wnt signal transduction pathway directs a variety of cell fate decisions in developing animal embryos. Despite the identification of many Wg pathway components to date, it is still not clear how these elements work together to generate cellular identities. In the ventral epidermis of Drosophila embryos, Wg specifies cells to secrete a characteristic pattern of denticles and naked cuticle that decorate the larval cuticle at the end of embryonic development. We have used the Drosophila ventral epidermis as our assay system in a series of genetic screens to identify new components involved in Wg signaling. Two mutant lines that modify wg-mediated epidermal patterning represent the first loss of function mutations in the RacGap50C gene. These mutations on their own cause increased stabilization of Armadillo and cuticle pattern disruptions that include replacement of ventral denticles with naked cuticle, which suggest that the mutant embryos suffer from ectopic Wg pathway activation. In addition, RacGap50C mutations interact genetically with naked cuticle and Axin, known negative regulators of the Wg pathway. These phenotypes suggest that the RacGap50C gene product participates in the negative regulation of Wg pathway activity.


http://www.genetics.org/cgi/content/abstract/161/4/1497

An F1 mapping population of the septoria tritici blotch pathogen of wheat, Mycosphaerella graminicola, was generated by crossing the two Dutch field isolates IPO323 and IPO94269. AFLP and RAPD marker data sets were combined to produce a high-density genetic linkage map. The final map contained 223 AFLP and 57 RAPD markers, plus the biological traits mating type and avirulence, in 23 linkage groups spanning 1216 cM. Many AFLPs and some RAPD markers were clustered. When markers were reduced to 1 per cluster, 229 unique positions were mapped, with an average distance of 5.3 cM between markers. Because M. graminicola probably has 17 or 18
chromosomes, at least 5 of the 23 linkage groups probably will need to be combined with others once additional markers are added to the map. This was confirmed by pulsed-field gel analysis; probes derived from 2 of the smallest linkage groups hybridized to two of the largest chromosome-sized bands, revealing a discrepancy between physical and genetic distance. The utility of the map was demonstrated by identifying molecular markers tightly linked to two genes of biological interest, mating type and avirulence. Bulked segregant analysis was used to identify additional molecular markers closely linked to these traits. This is the first genetic linkage map for any species in the genus Mycosphaerella or the family Mycosphaerellaceae.


http://www.genetics.org/cgi/content/abstract/164/2/685

The Louisiana iris species *Iris brevicaulis* and *I. fulva* are morphologically and karyotypically distinct yet frequently hybridize in nature. A group of high-copy-number TY3/gypsy-like retrotransposons was characterized from these species and used to develop molecular markers that take advantage of the abundance and distribution of these elements in the large iris genome. The copy number of these IRRE elements (for iris retroelement), is ~1 x 10^5, accounting for ~6-10% of the ~10,000-Mb haploid Louisiana iris genome. IRRE elements are transcriptionally active in *I. brevicaulis* and *I. fulva* and their F1 and backcross hybrids. The LTRs of the elements are more variable than the coding domains and can be used to define several distinct IRRE subfamilies. Transposon display or S-SAP markers specific to two of these subfamilies have been developed and are highly polymorphic among wild-collected individuals of each species. As IRRE elements are present in each of 11 iris species tested, the marker system has the potential to provide valuable comparative data on the dynamics of retrotransposition in large plant genomes.


http://www.genetics.org/cgi/content/abstract/165/2/721

A new mutation has arisen in a colony of mice transgenic for human (alpha)-galactosidase. The mutation is independent of the transgenic insertion, autosomal dominant, and morphologically very similar to the classical wavy coat mutation, caracul (Ca), on chromosome 15. Therefore, we designated this locus the caracul Rinshoken (CaRin). Applying a positional cloning approach, we identified the mK6irs1/Krt2-6g gene as a strong candidate for CaRin because among five Ca alleles examined mutations always occurred in the highly conserved positions of the (alpha)-helical rod domain (1A and 2B subdomain) of this putative gene product. The most striking finding is that four independently discovered alleles, the three preexistent alleles CaJ, Ca9J, Ca10J, and our allele CaRin, all share one identical amino acid deletion (N 140 del) and the fifth, CamedJ, has an amino acid substitution (A 431 D). These findings indicate that a mutation hotspot exists in the Ca locus. Additionally, we describe a Ca mutant allele induced by ENU mutagenesis, which also possesses an amino acid substitution (L 424 W) in the mK6irs1/Krt2-6g gene. The identification of the Ca candidate gene enables us to further define the nature of the genetic pathway required for hair formation and provides an important new candidate that may be implicated in human hair and skin diseases.

http://www.genetics.org/cgi/content/abstract/168/3/1655

Cf resistance genes in tomato confer resistance to the fungal leaf pathogen Cladosporium fulvum. Both the well-characterized resistance gene Cf-9 and the related 9DC gene confer resistance to strains of C. fulvum that secrete the Avr9 protein and originate from the wild tomato species Lycopersicon pimpinellifolium. We show that 9DC and Cf-9 are allelic, and we have isolated and sequenced the complete 9DC cluster of L. pimpinellifolium LA1301. This 9DC cluster harbors five full-length Cf homologs, including orthologs of the most distal homologs of the Cf-9 cluster and three central 9DC genes. Two 9DC genes (9DC1 and 9DC2) have an identical coding sequence, whereas 9DC3 differs at its 3' terminus. From a detailed comparison of the 9DC and Cf-9 clusters, we conclude that the Cf-9 and Hcr9-9D genes from the Cf-9 cluster are ancestral to the first 9DC gene and that the three 9DC genes were generated by subsequent intra- and intergenic unequal recombination events. Thus, the 9DC cluster has undergone substantial rearrangements in the central region, but not at the ends. Using transient transformation assays, we show that all three 9DC genes confer Avr9 responsiveness, but that 9DC2 is likely the main determinant of Avr9 recognition in LA1301.


http://www.genetics.org/cgi/content/abstract/167/4/1611

The influence of duplicated sequences on chromosomal stability is poorly understood. To characterize chromosomal rearrangements involving duplicated sequences, we compared the organization of tandem repeats of the DUP240 gene family in 15 Saccharomyces cerevisiae strains of various origins. The DUP240 gene family consists of 10 members of unknown function in the reference strain S288C. Five DUP240 paralogs on chromosome I and two on chromosome VII are arranged as tandem repeats that are highly polymorphic in copy number and sequence. We characterized DNA sequences that are likely involved in homologous or nonhomologous recombination events and are responsible for intra- and interchromosomal rearrangements that cause the creation and disappearance of DUP240 paralogs. The tandemly repeated DUP240 genes seem to be privileged sites of gene birth and death.


http://www.genetics.org/cgi/content/abstract/166/1/99

Race-cultivar specialization during the interaction of the basidiomycete smut pathogen Ustilago hordei with its barley host was described in the 1940s. Subsequent genetic analyses revealed the presence of dominant avirulence genes in the pathogen that conform to the gene-for-gene theory. This pathosystem therefore presents an opportunity for the molecular genetic characterization of fungal genes controlling avirulence. We performed a cross between U. hordei strains to obtain 54 progeny segregating for three dominant avirulence genes on three differential barley cultivars. Bulked segregant analysis was used to identify RAPD and AFLP markers tightly linked to the avirulence gene UhAvr1. The UhAvr1 gene is located in an area containing repetitive DNA and this region is undetectable in cosmid libraries prepared from the avirulent parental strain. PCR
and hybridization probes developed from the linked markers were therefore used to identify cosmid clones from the virulent (Uhavr1) parent. By walking on Uhavr1-linked cosmid clones, a nonrepetitive, nearby probe was found that recognized five overlapping BAC clones spanning 170 kb from the UhAvr1 parent. A contig of the clones in the UhAvr1 region was constructed and selected probes were used for RFLP analysis of the segregating population. This approach genetically defined an ~80-kb region that carries the UhAvr1 gene and provided cloned sequences for subsequent genetic analysis. UhAvr1 represents the first avirulence gene cloned from a basidiomycete plant pathogen.


http://www.genetics.org/cgi/content/abstract/165/1/47

The double-strand break repair (DSBR) model of recombination predicts that heteroduplexes will be formed in regions that flank the double-strand break (DSB) site and that the resulting intermediate is resolved to generate either crossovers or noncrossovers for flanking markers. Previous studies in Saccharomyces cerevisiae, however, failed to detect heteroduplexes on both sides of the DSB site. Recent physical studies suggest that some recombination events involve heteroduplex formation by a mechanism, synthesis-dependent strand annealing (SDSA), that is inherently asymmetric with respect to the DSB site and that leads exclusively to noncrossovers of flanking markers. Below, we demonstrate that many of the recombination events initiated at the HIS4 recombination hotspot are consistent with a variant of the DSBR model in which the extent of heteroduplex on one side of the DSB site is much greater than that on the other. Events that include only one flanking marker in the heteroduplex (unidirectional events) are usually resolved as noncrossovers, whereas events that include both flanking markers (bidirectional events) are usually resolved as crossovers. The unidirectional events may represent SDSA, consistent with the conclusions of others, although other possibilities are not excluded. We also show that the level of recombination reflects the integration of events initiated at several different DSB sites, and we identify a subset of gene conversion events that may involve break-induced replication (BIR) or repair of a double-stranded DNA gap.


http://www.genetics.org/cgi/content/abstract/161/1/143

Immature spermatids from Caenorhabditis elegans are stimulated by an external activation signal to reorganize their membranes and cytoskeleton to form crawling spermatozoa. This rapid maturation, termed spermiogenesis, occurs without any new gene expression. To better understand this signal transduction pathway, we isolated suppressors of a mutation in the spe-27 gene, which is part of the pathway. The suppressors bypass the requirement for spe-27, as well as three other genes that act in this pathway, spe-8, spe-12, and spe-29. Eighteen of the suppressor mutations are new alleles of spe-6, a previously identified gene required for an early stage of spermatogenesis. The original spe-6 mutations are loss-of-function alleles that prevent major sperm protein (MSP) assembly in the fibrous bodies of spermatocytes and arrest development in meiosis. We have isolated the spe-6 gene and find that it encodes a predicted protein-serine/threonine kinase in the casein kinase 1 family. The suppressor mutations appear to be reduction-of-function alleles. We propose a model whereby SPE-6, in addition to its early role in spermatocyte development, inhibits spermiogenesis until the activation signal is received. The activation signal is transduced through SPE-8, SPE-12, SPE-27, and SPE-29 to relieve SPE-6
repression, thus triggering the formation of crawling spermatozoa.


http://www.genetics.org/cgi/content/abstract/166/2/807

Genomic sequences provide powerful new tools in genetic analysis, making it possible to combine classical genetics with genomics to characterize the genes in a particular chromosome region. These approaches have been applied successfully to the euchromatin, but analysis of the heterochromatin has lagged somewhat behind. We describe a combined genetic and bioinformatics approach to the base of the right arm of the Drosophila melanogaster second chromosome, at the boundary between pericentric heterochromatin and euchromatin. We used resources provided by the genome project to derive a physical map of the region, examine gene density, and estimate the number of potential genes. We also carried out a large-scale genetic screen for lethal mutations in the region. We identified new alleles of the known essential genes and also identified mutations in 21 novel loci. Fourteen complementation groups map proximal to the assembled sequence. We used PCR to map the endpoints of several deficiencies and used the same set of deficiencies to order the essential genes, correlating the genetic and physical map. This allowed us to assign two of the complementation groups to particular "computed/curated genes" (CGs), one of which is Nipped-A, which our evidence suggests encodes Drosophila Tra1/TRRAP.


http://www.genetics.org/cgi/content/abstract/169/1/197

We studied microsatellite frequency and distribution in 21.76-Mb random genomic sequences, 0.67-Mb BAC sequences from the Z chromosome, and 6.3-Mb EST sequences of Bombyx mori. We mined microsatellites of \[\geq\]15 bases of mononucleotide repeats and \[\geq\]5 repeat units of other classes of repeats. We estimated that microsatellites account for 0.31% of the genome of B. mori. Microsatellite tracts of A, AT, and ATT were the most abundant whereas their number drastically decreased as the length of the repeat motif increased. In general, tri- and hexanucleotide repeats were overrepresented in the transcribed sequences except TAA, GTA, and TGA, which were in excess in genomic sequences. The Z chromosome sequences contained shorter repeat types than the rest of the chromosomes in addition to a higher abundance of AT-rich repeats. Our results showed that base composition of the flanking sequence has an influence on the origin and evolution of microsatellites. Transitions/transversions were high in microsatellites of ESTs, whereas the genomic sequence had an equal number of substitutions and indels. The average heterozygosity value for 23 polymorphic microsatellite loci surveyed in 13 diverse silkmoth strains having 2-14 alleles was 0.54. Only 36 (18.2%) of 198 microsatellite loci were polymorphic between the two divergent silkworm populations and 10 (5%) loci revealed null alleles. The microsatellite map generated using these polymorphic markers resulted in 8 linkage groups. B. mori microsatellite loci were the most conserved in its immediate ancestor, B. mandarina, followed by the wild saturniid silkmoth, Antheraea assama.

http://www.genetics.org/cgi/content/abstract/165/2/799

An RFLP genomic subtraction was used to isolate male-specific sequences in the species Silene latifolia. One isolated fragment, SLP2, shares similarity to a portion of the Activator (Ac) transposase from Zea mays and to related proteins from other plant species. Southern blot analysis of male and female S. latifolia genomic DNA shows that SLP2 belongs to a low-copy-number repeat family with two Y-linked copies. Screening of a S. latifolia male genomic library using SLP2 as a probe led to the isolation of five clones, which were partially sequenced. One clone contains two large open reading frames that can be joined into a sequence encoding a putative protein of 682 amino acids by removing a short intron. Database searches and phylogenetic analysis show that this protein belongs to the hAT superfamily of transposases, closest to Tag2 (Arabidopsis thaliana), and contains all of the defined domains critical for the activity of these transposases. PCR with genomic and cDNA templates from S. latifolia male, female, and hermaphrodite individuals revealed that one of the Y-linked copies is transcriptionally active and alternatively spliced. This is the first report of a transcriptionally active transposable element (TE) family in S. latifolia and the first DNA transposon residing on a plant Y chromosome. The potential activity and regulation of this TE family and its use for Y chromosome gene discovery is discussed.


http://www.genetics.org/cgi/content/abstract/166/1/307

The gray, short-tailed opossum, Monodelphis domestica, is the most extensively used, laboratory-bred marsupial resource for basic biologic and biomedical research worldwide. To enhance the research utility of this species, we are building a linkage map, using both anonymous markers and functional gene loci, that will enable the localization of quantitative trait loci (QTL) and provide comparative information regarding the evolution of mammalian and other vertebrate genomes. The current map is composed of 83 loci distributed among eight autosomal linkage groups and the X chromosome. The autosomal linkage groups appear to encompass a very large portion of the genome, yet span a sex-average distance of only 633.0 cM, making this the most compact linkage map known among vertebrates. Most surprising, the male map is much larger than the Female map (884.6 cM vs. 443.1 cM), a pattern contrary to that in eutherian mammals and other vertebrates. The finding of genome-wide reduction in female recombination in M. domestica, coupled with recombination data from two other, distantly related marsupial species, suggests that reduced female recombination might be a widespread metatherian attribute. We discuss possible explanations for reduced female recombination in marsupials as a consequence of the metatherian characteristic of determinate paternal X chromosome inactivation.


http://www.genetics.org/cgi/content/abstract/161/2/773
High levels of inheritable resistance to phosphine in Rhyzopertha dominica have recently been detected in Australia and in an effort to isolate the genes responsible for resistance we have used random amplified DNA fingerprinting (RAF) to produce a genetic linkage map of R. dominica. The map consists of 94 dominant DNA markers with an average distance between markers of 4.6 cM and defines nine linkage groups with a total recombination distance of 390.1 cM. We have identified two loci that are responsible for high-level resistance. One provides \([\sim]50x\) resistance to phosphine while the other provides 12.5x resistance and in combination, the two genes act synergistically to provide a resistance level 250x greater than that of fully susceptible beetles. The haploid genome size has been determined to be 4.76 x 10^8 bp, resulting in an average physical distance of 1.2 Mbp per map unit. No recombination has been observed between either of the two resistance loci and their adjacent DNA markers in a population of 44 fully resistant F5 individuals, which indicates that the genes are likely to reside within 0.91 cM (1.1 Mbp) of the DNA markers.


http://www.genetics.org/cgi/content/abstract/169/3/1403

Loline alkaloids are produced by mutualistic fungi symbiotic with grasses, and they protect the host plants from insects. Here we identify in the fungal symbiont, Neotyphodium uncinatum, two homologous gene clusters (LOL-1 and LOL-2) associated with loline-alkaloid production. Nine genes were identified in a 25-kb region of LOL-1 and designated (in order) lolF-1, lolC-1, lolD-1, lolO-1, lolA-1, lolU-1, lolP-1, lolT-1, and lolE-1. LOL-2 contained the homologs lolC-2 through lolE-2 in the same order and orientation. Also identified was lolF-2, but its possible linkage with either cluster was undetermined. Most lol genes were regulated in *N. uncinatum* and *N. coenophialum*, and all were expressed concomitantly with loline-alkaloid biosynthesis. A lolC-2 RNA-interference (RNAi) construct was introduced into *N. uncinatum*, and in two independent transformants, RNAi significantly decreased lolC expression (P < 0.01) and loline-alkaloid accumulation in culture (P < 0.001) compared to vector-only controls, indicating involvement of lolC in biosynthesis of lolines. The predicted LolU protein has a DNA-binding site signature, and the relationships of other lol-gene products indicate that the pathway has evolved from various different primary and secondary biosynthesis pathways.


http://www.genetics.org/cgi/content/abstract/164/1/259

The rate of mutation for nucleotide substitution is generally higher among males than among females, likely owing to the larger number of DNA replications in spermatogenesis than in oogenesis. For insertion and deletion (indel) mutations, data from a few human genetic disease loci indicate that the two sexes may mutate at similar rates, possibly because such mutations arise in connection with meiotic crossing over. To address origin- and sex-specific rates of indel mutation we have conducted the first large-scale molecular evolutionary analysis of indels in noncoding DNA sequences from sex chromosomes. The rates are similar on the X and Y chromosomes of primates but about twice as high on the avian Z chromosome as on the W chromosome. The fact that indels are not uncommon on the nonrecombining Y and W chromosomes excludes meiotic crossing over as the main cause of indel mutation. On the other hand, the similar rates on X and Y indicate that the number of DNA replications (higher for Y than for X) is also not the main factor. Our observations are therefore consistent with a role of both DNA replication and recombination in the generation of short insertion and deletion mutations.
significant excess of deletion compared to insertion events is observed on the avian W chromosome, consistent with gradual DNA loss on a nonrecombining chromosome.


http://www.genetics.org/cgi/content/abstract/167/1/377

Understanding the population genetic factors that shape genome variability is pivotal to the design and interpretation of studies using large-scale polymorphism data. We analyzed patterns of polymorphism and divergence at Z-linked and autosomal loci in the domestic chicken (Gallus gallus) to study the influence of mutation, effective population size, selection, and demography on levels of genetic diversity. A total of 14 autosomal introns (8316 bp) and 13 Z-linked introns (6856 bp) were sequenced in 50 chicken chromosomes from 10 highly divergent breeds. Genetic variation was significantly lower at Z-linked than at autosomal loci, with one segregating site every 39 bp at autosomal loci \((\theta_W = 5.8 \pm 0.8 \times 10^{-3})\) and one every 156 bp on the Z chromosome \((\theta_W = 1.4 \pm 0.4 \times 10^{-3})\). This difference may in part be due to a low male effective population size arising from skewed reproductive success among males, evident both in the wild ancestor—the red jungle fowl—and in poultry breeding. However, this effect cannot entirely explain the observed three- to fourfold reduction in Z chromosome diversity. Selection, in particular selective sweeps, may therefore have had an impact on reducing variation on the Z chromosome, a hypothesis supported by the observation of heterogeneity in diversity levels among loci on the Z chromosome and the lower recombination rate on Z than on autosomes. Selection on sex-linked genes may be particularly important in organisms with female heterogamety since the heritability of sex-linked sexually antagonistic alleles advantageous to males is improved when fathers pass a Z chromosome to their sons.


http://www.genetics.org/cgi/content/abstract/168/1/215

Colias eurytheme and C. philodice are sister species with broad sympatry in North America. They hybridize frequently and likely share a significant portion of their genomes through introgression. Both taxa have been ecologically well characterized and exploited to address a broad spectrum of evolutionary issues. Using AFLP markers, we constructed the first linkage map of Colias butterflies. The map is composed of 452 markers spanning 2541.7 cM distributed over 51 linkage groups (40 major groups and 11 small groups with 2-4 markers). Statistical tests indicate that these AFLP markers tend to cluster over the map, with the coefficient of variation of interval sizes being 1.236 (95% C.I. is 1.234-1.240). This nonrandom marker distribution can account for the nonequivalence between the number of linkage groups and the actual haploid chromosome number \((N = 31)\). This study presents the initial step for further marker-assisted research on Colias butterflies, including QTL and introgression analyses. Further investigation of the genomes will help us understand better the roles of introgression and natural selection in the evolution of hybridizing species and devise more appropriate strategies to control these pests.

The cosQ site of bacteriophage λ is required for DNA packaging termination. Previous studies have shown that cosQ mutations can be suppressed in three ways: by a local suppressor within cosQ, an increase in the length of the λ chromosome, and missense mutations affecting the prohead’s portal protein, gpB. In the present work, revertants of a set of lethal cosQ mutants were screened for suppressors. Seven new cosQ suppressors affected gene B, which encodes the portal protein of the prohead. All seven were allele-nonspecific suppressors of cosQ mutations. Experiments with several phages having two cosQ suppressors showed that the suppression effects were additive. Furthermore, these double suppressors had minimal effects on the growth of cosQ+ phages. These trans-acting suppressors affecting the portal protein are proposed to allow the mutant cosQ site to be more efficiently recognized, due to the slowing of the rate of translocation.

Genome Res.  (26)


LTR-containing retrotransposons reverse transcribe their RNA genomes, and the resulting cDNAs are integrated into the genome by the element-encoded integrase protein. The yeast LTR retrotransposon Ty1 preferentially integrates into a target window upstream of tDNAs (tRNA genes) in the yeast genome. We investigated the nature of these insertions and the target window on a genomic scale by analyzing several hundred de novo insertions upstream of tDNAs in two different multicopy gene families. The pattern of insertion upstream of tDNAs was nonrandom and periodic, with peaks separated by ~80 bp. Insertions were not distributed equally throughout the genome, as certain tDNAs within a given family received higher frequencies of upstream Ty1 insertions than others. We showed that the presence and relative position of additional tDNAs and LTRs surrounding the target tDNA dramatically influenced the frequency of insertion events upstream of that target.


Inversions breaking the 1041 bp int1h-1 or the 9.5-kb int22h-1 sequence of the F8 gene cause hemophilia A in 1/30,000 males. These inversions are due to homologous recombination between the above sequences and their inverted copies on the same DNA molecule, respectively, int1h-2 and int22h-2 or int22h-3. We find that (1) int1h and int22h duplicated more than 25 million years ago; (2) the identity of the copies (>99%) of these sequences in humans and other primates is due to gene conversion; (3) gene conversion is most frequent in the internal regions of int22h; (4) breakpoints of int22h-related inversions also tend to involve the internal regions of int22h; (5) sequence variations in a sample of human X chromosomes defined eight haplotypes of int22h-1 and 27 of int22h-2 plus int22h-3; (6) the latter two sequences, which lie, respectively, 500 and
600 kb telomeric to int22h-1 are five-fold more identical when in cis than when in trans, thus suggesting that gene conversion may be predominantly intrachromosomal; (7) int1h, int22h, and flanking sequences evolved at a rate of about 0.1% substitutions per million years during the divergence between humans and other primates, except for int1h during the human-chimpanzee divergence, when its rate of evolution was significantly lower. This is reminiscent of the slower evolution of palindrome arms in the male specific regions of the Y chromosome and we propose, as an explanation, that intrachromosomal gene conversion and cosegregation of the duplicated regions favors retention of the ancestral sequence and thus reduces the evolution rate.


http://www.genome.org/cgi/content/abstract/14/2/287

Genomic copy number alterations are a feature of many human diseases including cancer. We have evaluated the effectiveness of an oligonucleotide array, originally designed to detect single-nucleotide polymorphisms, to assess DNA copy number. We first showed that fluorescent signal from the oligonucleotide array varies in proportion to both decreases and increases in copy number. Subsequently we applied the system to a series of 20 cancer cell lines. All of the putative homozygous deletions (10) and high-level amplifications (12; putative copy number >4) tested were confirmed by PCR (either qPCR or normal PCR) analysis. Low-level copy number changes for two of the lines under analysis were compared with BAC array CGH; 77% (n = 44) of the autosomal chromosomes used in the comparison showed consistent patterns of LOH (loss of heterozygosity) and low-level amplification. Of the remaining 10 comparisons that were discordant, eight were caused by low SNP densities and failed in both lines. The studies demonstrate that combining the genotype and copy number analyses gives greater insight into the underlying genetic alterations in cancer cells with identification of complex events including loss and reduplication of loci.


http://www.genome.org/cgi/content/abstract/13/5/925

We report the validation of a new assay for typing single nucleotide polymorphisms (SNPs) that takes advantage of the 3'-to-5' exonuclease proofreading activity of many DNA polymerases. The assay uses one or more primers labeled on the 3' nucleotide base, and can be implemented in a variety of formats including a one-step PCR reaction that allows SNP typing directly from genomic DNA samples. The detection of genotypes can be accomplished by means of fluorescence detection on assays that have been purified to remove excess primer, or by means of fluorescence polarization without any additional cleanup. We also demonstrate that the Exo-Proofreading SNP assay can be used on pooled samples to obtain allele frequency data.


http://www.genome.org/cgi/content/abstract/12/3/414

Nuclear microsatellite loci (2- to 5-bp tandem repeats) would seem to be ideal markers for
population genetic monitoring because of their abundant polymorphism, wide dispersal in vertebrate genomes, near selective neutrality, and ease of assessment; however, questions about their mode of generation, mutation rates and ascertainment bias have limited interpretation considerably. We have assessed the patterns of genomic diversity for ninety feline microsatellite loci among previously characterized populations of cheetahs, lions and pumas in recapitulating demographic history. The results imply that the microsatellite diversity measures (heterozygosity, allele reconstitution and microsatellite allele variance) offer proportionate indicators, albeit with large variance, of historic population bottlenecks and founder effects. The observed rate of reconstruction of new alleles plus the growth in the breadth of microsatellite allele size (variance) was used here to develop genomic estimates of time intervals following historic founder events in cheetahs (12,000 yr ago), in North American pumas (10,000-17,000 yr ago), and in Asiatic lions of the Gir Forest (1000-4000 yr ago).[Supplemental material available online at http://rex.nci.nih.gov/lgd/front_page.htm and at http://www.genome.org.]


http://www.genome.org/cgi/content/abstract/12/9/1428

A DNA mutation detection protocol able to identify and characterize a previously unknown change in a given sequence in a rapid, efficient, sensitive, and inexpensive manner is required to take advantage of the resources now available to researchers through the genome sequencing projects. We have developed a method based on base-specific cleavage of polymerase chain reaction (PCR) products and then separation of the fragments by matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), which can meet these criteria. Differences are seen as the presence, absence, or mass change of peaks corresponding to fragments affected by the base difference. This technique is shown through the detection of a polymorphism in the 3' untranslated region of IL12p40 from a double-stranded PCR product, and the detection of a single nucleotide polymorphism between two mouse strains. The sensitivity of the technique can be increased with the use of postsource decay, which enables differentiation of two fragments of identical mass but different sequence. The level of specificity and the rapid sample analysis time lend this technique to the mass screening of individuals for sequence changes and, in combination with MS sequencing methods, could be used to facilitate rapid resequencing of DNA.


http://www.genome.org/cgi/content/abstract/12/11/1651

Human chromosome 2 was formed by the head-to-head fusion of two ancestral chromosomes that remained separate in other primates. Sequences that once resided near the ends of the ancestral chromosomes are now interstitially located in 2q13-2q14.1. Portions of these sequences had duplicated to other locations prior to the fusion. Here we present analyses of the genomic structure and evolutionary history of >600 kb surrounding the fusion site and closely related sequences on other human chromosomes. Sequence blocks that closely flank the inverted arrays of degenerate telomere repeats marking the fusion site are duplicated at many, primarily subtelomeric, locations. In addition, large portions of a 168-kb centromere-proximal block are duplicated at 9pter, 9p11.2, and 9q13, with 98%-99% average sequence identity. A 67-kb block on the distal side of the fusion site is highly homologous to sequences at 22qter. A third ~100-kb segment is 96% identical to a region in 2q11.2. By integrating data on the extent and similarity of these paralogous blocks, including the presence of phylogenetically informative
repetitive elements, with observations of their chromosomal distribution in nonhuman primates, we infer the order of the duplications that led to their current arrangement. Several of these duplicated blocks may be associated with breakpoints of inversions that occurred during primate evolution and of recurrent chromosome rearrangements in humans.[Supplemental material is available online at http://www.genome.org. The following individuals kindly provided reagents, samples, or unpublished information as indicated in the paper: T. Newman, C. Harris, and J. Young.]


http://www.genome.org/cgi/content/abstract/12/11/1663

Various portions of the region surrounding the site where two ancestral chromosomes fused to form human chromosome 2 are duplicated elsewhere in the human genome, primarily in subtelomeric and pericentromeric locations. At least 24 potentially functional genes and 16 pseudogenes reside in the 614-kb of sequence surrounding the fusion site and paralogous segments on other chromosomes. By comparing the sequences of genomic copies and transcripts, we show that at least 18 of the genes in these paralogous regions are transcriptionally active. Among these genes are new members of the cobalamin synthetase W domain (CBWD) and forkhead domain FOXD4 gene families. Copies of RPL23A and SNRPA1 on chromosome 2 are retrotransposed-processed pseudogenes that were included in segmental duplications; we find 53 RPL23A pseudogenes in the human genome and map the functional copy of SNRPA1 to 15pter. The draft sequence of the human genome also provides new information on the location and intron-exon structure of functional copies of other 2q-fusion genes (PGM5, retina-specific F379, helicase CHLR1, and acrosin). This study illustrates that the duplication and rearrangement of subtelomeric and pericentromeric regions have functional relevance to human biology; these processes can change gene dosage and/or generate genes with new functions.[Supplemental material is available online at http://www.genome.org. Sequence data reported in this paper have been deposited in GenBank and assigned the following accession nos.: AF452722, AF452723, and AF452724.]


http://www.genome.org/cgi/content/abstract/12/9/1357

Olfaction is of considerable importance to many insects in behaviors critical for survival and reproduction, including location of food sources, selection of mates, recognition of colony con-specifics, and determination of oviposition sites. An ubiquitous, but poorly understood, component of the insect's olfactory system is a group of odorant-binding proteins (OBPs) that are present at high concentrations in the aqueous lymph surrounding the dendrites of olfactory receptor neurons. OBPs are believed to shuttle odorants from the environment to the underlying odorant receptors, for which they could potentially serve as odorant presenters. Here we show that the Drosophila genome carries 51 potential OBP genes, a number comparable to that of its odorant-receptor genes. We find that the majority (73%) of these OBP-like genes occur in clusters of as many as nine genes, in contrast to what has been observed for the Drosophila odorant-receptor genes. Two of the presumptive OBP gene clusters each carries an odorant-receptor gene. We also report an intriguing subfamily of 12 putative OBPs that share a unique C-terminal structure with three conserved cysteines and a conserved proline. Members of this subfamily have not previously been described for any insect. We have performed phylogenetic analyses of the OBP-
related proteins in Drosophila as well as other insects, and we discuss the duplication and divergence of the genes for this large family.[The sequence data from this study have been submitted to FlyBase. Annotations for these sequences are available as supplementary material at http://www.genome.org.]


http://www.genome.org/cgi/content/abstract/12/9/1401

Fluorescence resonance energy transfer (FRET) is a powerful tool for detecting spatial relationships between macromolecules, one use of which is the tracking of DNA hybridization status. The process involves measuring changes in fluorescence as FRET donor and acceptor moieties are brought closer together or moved farther apart as a result of DNA hybridization/denaturation. In the present study, we introduce a new version of FRET, which we term induced FRET (iFRET), that is ideally suited for melting curve analysis. The innovation entails using a double-strand, DNA-specific intercalating dye (e.g., SYBR Green I) as the FRET donor, with a conventional FRET acceptor affixed to one of the DNA molecules. The SNP genotyping technique dynamic allele specific hybridization (DASH) was used as a platform to compare iFRET to two alternative fluorescence strategies, namely, the use of the intercalating dye alone and the use of a standard FRET pair (fluorescein as donor, 6-rhodamine as acceptor). The iFRET configuration combines the advantages of intercalating dyes, such as high signal strengths and low cost, with maintaining the specificity and multiplex potential afforded by traditional FRET detection systems. Consequently, iFRET represents a fresh and attractive schema for monitoring interactions between DNA molecules.


http://www.genome.org/cgi/content/abstract/13/5/916

Genotyping technologies need to be continually improved in terms of their flexibility, cost-efficiency, and throughput, to push forward genome variation analysis. To this end, we have leveraged the inherent simplicity of dynamic allele-specific hybridization (DASH) and coupled it to recent innovations of centrifugal arrays and iFRET. We have thereby created a new genotyping platform we term DASH-2, which we demonstrate and evaluate in this report. The system is highly flexible in many ways (any plate format, PCR multiplexing, serial and parallel array processing, spectral-multiplexing of hybridization probes), thus supporting a wide range of application scales and objectives. Precision is demonstrated to be in the range 99.8-100%, and assay costs are 0.05 USD or less per genotype assignment. DASH-2 thus provides a powerful new alternative for genotyping practice, which can be used without the need for expensive robotics support.


http://www.genome.org/cgi/content/abstract/12/6/985
Messenger RNAs that have the stability determinants, adenylate uridylate-rich elements (AREs), in their 3’ untranslated region (UTR) code for key products that regulate early and transient biological responses. We used a computational laboratory approach for amplification of large, including full-length, protein-coding regions for ARE genes. Statistical analysis of the initiation regions in the 5’ UTR of ARE-mRNAs was performed. Accordingly, several 5’ primers and a single universal 3’ primer that targeted the initiation consensuses and ARE regions, respectively, were designed. Using optimized conditions, the primers were able to enrich and amplify large protein-coding regions for the ARE gene family. The selective amplification of ARE cDNAs was verified using specific polymerase chain reactions (PCRs) to known ARE mRNA molecules and monitoring the abundance of the non-ARE [beta]-actin signal. A mini-library from the amplified ARE products was constructed for further confirmation of ARE selection. Distinct ARE amplified cDNA pools were selectively generated by distinct 5’ primers. The biological utility of the method was shown with differential display. The up-regulation of several ARE-mRNAs, including the full-length coding region of the small inducible cytokine A4 (SCYA4) gene, was shown in endotoxin-stimulated monocytic cells. The integrated computational and laboratory approach should lead to enhanced capability for discovery and expression analysis of early and transient response genes.


http://www.genome.org/cgi/content/abstract/13/8/1966

As a step toward the goal of adding the cattle genome to those available for multispecies comparative genome analysis, 40,224 cattle BAC clones were end-sequenced, yielding 60,547 sequences (BAC end sequences, BESs) after trimming with an average read length of 515 bp. Cattle BACs were anchored to the human and mouse genome sequences by BLASTN search, revealing 29.4% and 10.1% significant hits (E < e-5), respectively. More than 60% of all cattle BES hits in both the human and mouse genomes are located within known genes. In order to confirm in silico predictions of orthology and their relative position on cattle chromosomes, 84 cattle BESs with similarity to sequences on HSA11 were mapped using a cattle-hamster radiation hybrid (RH) panel. Resulting RH maps of BTA15 and BTA29 cover [~]85% of HSA11 sequence, revealing a complex patchwork shuffling of segments not explained by a simple translocation followed by internal rearrangements. Overlay of the mouse conserved syntenies onto HSA11 revealed that segmental boundaries appear to be conserved in all three species. The BAC clone-based comparative map provides a foundation for the evolutionary analysis of mammalian karyotypes and for sequencing of the cattle genome.


http://www.genome.org/cgi/content/abstract/12/12/1885

Fish-odor syndrome or Trimethylaminuria (OMIM #602079) in humans is an inborn error of metabolism associated with a characteristic fishy body odor due to elevated levels of trimethylamine (TMA) in body fluids. It is caused by loss-of-function mutations in FMO3 encoding flavin-containing mono-oxygenase 3. A fishy off-flavor is occasionally observed in cow’s milk and it has been established recently that this phenotype is due to elevated TMA levels. Here, we report that fishy off-flavor in cow’s milk is caused by a nonsense mutation (R238X) in the bovine FMO3 ortholog. RT-PCR analysis indicated that the mutant transcript is present in a very low amount. The mutation was found to be surprisingly common (q = 0.155) in one breed of cattle.[The sequence data described in this paper have been submitted to GenBank with accession nos. AF488417-AF488422. The following individuals kindly provided reagents,
The analysis of single nucleotide polymorphisms (SNPs) is increasingly utilized to investigate the genetic causes of complex human diseases. Here we present a high-throughput genotyping platform that uses a one-primer assay to genotype over 10,000 SNPs per individual on a single oligonucleotide array. This approach uses restriction digestion to fractionate the genome, followed by amplification of a specific fractionated subset of the genome. The resulting reduction in genome complexity enables allele-specific hybridization to the array. The selection of SNPs was primarily determined by computer-predicted lengths of restriction fragments containing the SNPs, and was further driven by strict empirical measurements of accuracy, reproducibility, and average call rate, which we estimate to be >9.5%, >99.9%, and >95%, respectively. With average heterozygosity of 0.38 and genome scan resolution of 0.31 cM, the SNP array is a viable alternative to panels of microsatellites (STRs). As a demonstration of the utility of the genotyping platform in whole-genome scans, we have replicated and refined a linkage region on chromosome 2p for chronic mucocutaneous candidiasis and thyroid disease, previously identified using a panel of microsatellite (STR) markers.


http://www.genome.org/cgi/content/abstract/14/3/478

We report 80,388 ESTs from 23 Atlantic salmon (Salmo salar) cdNA libraries (61,819 ESTs), 6 rainbow trout (Oncorhynchus mykiss) cdNA libraries (14,544 ESTs), 2 chinook salmon (Oncorhynchus tshawytscha) cdNA libraries (1317 ESTs), 2 sockeye salmon (Oncorhynchus nerka) cdNA libraries (1243 ESTs), and 2 lake whitefish (Coregonus clupeaformis) cdNA libraries (1465 ESTs). The majority of these are 3’ sequences, allowing discrimination between paralogs arising from a recent genome duplication in the salmonid lineage. Sequence assembly reveals 28,710 different S. salar, 8981 O. mykiss, 1085 O. tshawytscha, 520 O. nerka, and 1176 C. clupeaformis putative transcripts. We annotate the submitted portion of our EST database by molecular function. Higher- and lower-molecular-weight fractions of libraries are shown to contain distinct gene sets, and higher rates of gene discovery are associated with higher-molecular weight libraries. Pyloric caecum library group annotations indicate this organ may function in redox control and as a barrier against systemic uptake of xenobiotics. A microarray is described, containing 7356 salmonid elements representing 3557 different cdNA sequences. Analyses of cross-species hybridizations to this cdNA microarray indicate that this resource may be used for studies involving all salmonids.


http://www.genome.org/cgi/content/abstract/13/1/122
We have developed a unique comprehensive mouse radiation hybrid (RH) map of nearly 23,000 markers integrating data from three international genome centers and over 400 independent laboratories. We have cross-referenced this map to the 0.5-cM resolution recombination-based Jackson Laboratory (TJL) backcross panel map, building a complete set of RH framework chromosome maps based on a high density of known-ordered anchor markers. We have systematically typed markers to improve coverage and resolve discrepancies, and have reanalyzed data sets as needed. The cross-linking of the RH and recombination maps has resulted in a highly accurate genome-wide map with consistent marker order. We have compared these linked framework maps to the Ensembl mouse genome sequence assembly, and show that they are a useful medium resolution tool for both validating sequence assembly and elucidating chromosome biology. [Supplemental material is available online at www.genome.org.]


http://www.genome.org/cgi/content/abstract/14/2/267

Lahn and Page previously observed that genes on the human X chromosome were physically arranged along the chromosome in "strata," roughly ordered by degree of divergence from related genes on the Y chromosome. They hypothesized that this ordering results from a historical series of suppressions of recombination along the mammalian Y chromosome, thereby allowing formerly recombining X and Y chromosomal genes to diverge independently. Here predictions of this hypothesis are confirmed in a nonprimate mammalian order, Rodentia, through an analysis of eight gene pairs from the X and Y chromosomes of the house mouse, Mus musculus. The mouse X chromosome has been rearranged relative to the human X, so strata were not found in the same physical order on the mouse X. However, based on synonymous evolutionary distances, X-linked genes in M. musculus fall into the same strata as orthologous genes in humans, as predicted. The boundary between strata 2 and 3 is statistically significant, but the boundary between strata 1 and 2 is not significant in mice. An analysis of smaller fragments of Smcy, Smcx, Zfy, and Zfx from seven species of Mus confirmed that the strata in Mus musculus were representative of the genus Mus.


http://www.genome.org/cgi/content/abstract/13/8/1944

Peptide mass-signature genotyping (PMSG) is a scanning genotyping method that identifies mutations and polymorphisms by translating the sequence of interest in more than one reading frame and measuring the masses of the resulting peptides by mass spectrometry. PMSG was applied to the RDS/peripherin gene of 16 individuals from a family exhibiting autosomal dominant macular degeneration. The method revealed an A-[gt]T transversion in the 5’ splice site of intron 2 that is the likely cause of the disease. It also revealed four different minihaplotypes in exon 3 that represent particular combinations of SNPs at four different locations. This study demonstrates the utility of PMSG for identifying and characterizing point mutations and local minihaplotypes that are not readily analyzed by other approaches.

Despite recent advances in linear whole genome amplification of intact DNA/RNA, amplification of degraded nucleic acids in an unbiased fashion remains a serious challenge for genetic diagnosis. We describe a new whole genome amplification procedure, RCA-RCA (Restriction and Circularization-Aided Rolling Circle Amplification), which retains the allelic differences among degraded amplified genomes while achieving almost complete genome coverage. RCA-RCA utilizes restriction digestion and whole genome circularization to generate genomic sequences amenable to rolling circle amplification. When intact genomic DNA is used, RCA-RCA retains gene-amplification differences (twofold or higher) between complex genomes on a genome-wide scale providing highly improved concordance with unamplified material as compared with other amplification methodologies including multiple displacement amplification. Using RCA-RCA, formalin-fixed samples of modest or substantial DNA degradation were successfully amplified and screened via array-CGH or Taqman PCR that displayed retention of the principal gene amplification features of the original material. Microsatellite analysis revealed that RCA-RCA amplified genomic DNA is representative of the original material at the nucleotide level. Amplification of cDNA is successfully performed via RCA-RCA and results to unbiased gene expression analysis ($R^2 = 0.99$). The simplicity and universal applicability of RCA-RCA make it a powerful new tool for genome analysis with unique advantages over previous amplification technologies.


To accelerate the molecular analysis of behavior in the honey bee (Apis mellifera), we created expressed sequence tag (EST) and cDNA microarray resources for the bee brain. Over 20,000 cDNA clones were partially sequenced from a normalized (and subsequently subtracted) library generated from adult A. mellifera brains. These sequences were processed to identify 15,311 high-quality ESTs representing 8912 putative transcripts. Putative transcripts were functionally annotated (using the Gene Ontology classification system) based on matching gene sequences in Drosophila melanogaster. The brain ESTs represent a broad range of molecular functions and biological processes, with neurobiological classifications particularly well represented. Roughly half of Drosophila genes currently implicated in synaptic transmission and/or behavior are represented in the Apis EST set. Of Apis sequences with open reading frames of at least 450 bp, 24% are highly diverged with no matches to known protein sequences. Additionally, over 100 Apis transcript sequences conserved with other organisms appear to have been lost from the Drosophila genome. DNA microarrays were fabricated with over 7000 EST cDNA clones putatively representing different transcripts. Using probe derived from single bee brain mRNA, microarrays detected gene expression for 90% of Apis cDNAs two standard deviations greater than exogenous control cDNAs. [The sequence data described in this paper have been submitted to Genbank data library under accession nos. BI502708-BI517278. The sequences are also available at http://titan.biotec.uiuc.edu/bee/honeybee_project.htm.]

The analysis of human genetic variations such as single nucleotide polymorphisms (SNPs) has great applications in genome-wide association studies of complex genetic traits. We have developed an SNP genotyping method based on the primer extension assay with fluorescence quenching as the detection. The template-directed dye-terminator incorporation with fluorescence quenching detection (FQ-TDI) assay is based on the observation that the intensity of fluorescent dye R110- and R6G-labeled acycloterminators is universally quenched once they are incorporated onto a DNA oligonucleotide primer. By comparing the rate of fluorescence quenching of the two allelic dyes in real time, we have extended this method for allele frequency estimation of SNPs in pooled DNA samples. The kinetic FQ-TDI assay is highly accurate and reproducible both in genotyping and in allele frequency estimation. Allele frequencies estimated by the kinetic FQ-TDI assay correlated well with known allele frequencies, with an r² value of 0.993. Applying this strategy to large-scale studies will greatly reduce the time and cost for genotyping hundreds and thousands of SNP markers between affected and control populations.


http://www.genome.org/cgi/content/abstract/13/7/1754

We report here a new mechanism for allelic discrimination--allele-specific Holliday Junction formation. The Holliday Junction (HJ) is a unique DNA structure that can be formed in a sequence-nonspecific manner by routine PCR. To cause the PCR-based HJ formation to occur in an allele-specific manner, the PCR primers are manipulated such that an extra mismatch next to a SNP of interest is introduced between a target and a reference amplicon and a GC-clamp is added. Based on this new mechanism, novel SNP genotyping methods were developed, including a homogeneous fluorescence polarization (FP) competition assay that requires neither labeled primers/probes nor expensive enzymes/substrates. Using this novel genotyping technology, we were able to convert >95% of SNP sequences into genotyping assays that work well under a universal set of assay conditions and achieved 100% accuracy in clinical samples.


http://www.genome.org/cgi/content/abstract/15/4/505

Comparative genome analysis is a powerful tool that can facilitate the reconstruction of the evolutionary history of the genomes of modern-day species. The model plant Arabidopsis thaliana with its n = 5 genome is thought to be derived from an ancestral n = 8 genome. Pairwise comparative genome analyses of A. thaliana with polyploid and diploid Brassicaceae species have suggested that rapid genome evolution, manifested by chromosomal rearrangements and duplications, characterizes the polyploid, but not the diploid, lineages of this family. In this study, we constructed a low-density genetic linkage map of Arabidopsis lyrata ssp. lyrata (A. l. lyrata; n = 8, diploid), the closest known relative of A. thaliana (MRCA [-]5 Mya), using A. thaliana-specific markers that resolve into the expected eight linkage groups. We then performed comparative Bayesian analyses using raw mapping data from this study and from a Capsella study to infer the number and nature of rearrangements that distinguish the n = 8 genomes of A. l. lyrata and Capsella from the n = 5 genome of A. thaliana. We conclude that there is strong statistical support in favor of the parsimony scenarios of 10 major chromosomal rearrangements separating these n = 8 genomes from A. thaliana. These chromosomal rearrangement events contribute to a rate of chromosomal evolution higher than previously reported in this lineage. We infer that at least seven of these events, common to both sets of data, are responsible for the change in
karyotype and underlie genome reduction in A. thaliana.


http://www.genome.org/cgi/content/abstract/12/3/357

The forced swim test (FST) and tail suspension test (TST) are widely used and well established screening paradigms for antidepressants. A variety of antidepressive agents are known to reduce immobility time in both FST and TST. To identify genetic determinants of immobility duration in both tests, we analyzed 560 F2 mice from an intercross between C57BL/6 (B6) and C3H/He (C3) strains. Composite interval mapping revealed five major loci (suggestive and significant linkage) affecting immobility in the FST, and four loci for the TST. The quantitative trait loci (QTL) on chromosomes 8 and 11 overlap between the two behavioral measures. Genome-wide interaction analysis, which was developed to identify locus pairs that may contribute epistatically to a phenotype, detected two pairs of chromosomal loci for the TST. The QTL on chromosome 11 and its associated epistatic TST-QTL on chromosome X encode [gamma]-aminobutyric acid type A (GABAA) receptor subunits as candidates. Sequence and expression analyses of these genes from the two parental strains revealed a significantly lower expression of the [alpha]1 subunit gene in the frontal cortex of B6 mice compared to C3 mice. The present quantitative trait study should open up avenues for identifying novel molecular targets for antidepressants and unravelling the complex genetic mechanisms of depressive and anxiety disorders.


http://www.genome.org/cgi/content/abstract/13/2/173

Association studies of candidate genes with complex traits have generally used one or a few single nucleotide polymorphisms (SNPs), although variation in the extent of linkage disequilibrium (LD) within genes markedly influences the sensitivity and precision of association studies. The extent of LD and the underlying haplotype structure for most candidate genes are still unavailable. We sampled 193 blacks (African-Americans) and 160 whites (European-Americans) and estimated the intragenic LD and the haplotype structure in four genes of the renin-angiotensin system. We genotyped 25 SNPs, with all but one of the pairs spaced between 1 and 20 kb, thus providing resolution at small scale. The pattern of LD within a gene was very heterogeneous. Using a robust method to define haplotype blocks, blocks of limited haplotype diversity were identified at each locus; between these blocks, LD was lost owing to the history of recombination events. As anticipated, there was less LD among blacks, the number of haplotypes was substantially larger, and shorter haplotype segments were found, compared with whites. These findings have implications for candidate-gene association studies and indicate that variation between populations of European and African origin in haplotype diversity is characteristic of most genes. [The sequence data described in this paper are available in GenBank under the following accession nos: AGT, MIM 106150; Renin, MIM 179820; ACE, MIM 106180; Angiotensin receptor I, MIM 106165. Supplementary material is available online at http://www.genome.org.]

http://www.sciencedirect.com/science/article/B6V67-47YPP75-3/2/dc591b0e0e66d996568adb82f9ab2447c

Rapid methods for characterizing soil microbial communities are essential to assess responses to perturbations and to improved management practices. This study compared the composition of microbial communities in 47 agricultural soil and adjacent land use samples collected in the San Joaquin Valley, CA. Microbial communities were characterized by DNA fingerprinting of the Intergenic Transcribed Spacer (ITS) region, using primers universal for bacteria or eucarya. Bacterial DNA fingerprints were more complex (containing 25-30 bands) than were eucaryotic fingerprints (8-15 bands). Field replicates from within an agricultural field were more similar to one another than samples collected in different fields under the same crop type or in close proximity to one another. Microbial communities in almond, grape, and tomato soils across different locations were more similar to one another than communities in cotton and safflower soils. Bacterial DNA fingerprints were significantly correlated with soil electrical conductivity, soil texture, inorganic carbon, and nitrogen content but not with pH and organic carbon content. The grouping of soil samples based on their soil reflectance properties was similar to the grouping based on the bacterial ITS analysis. Despite similarities among communities under some crops and at some locations, there is tremendous unexplained diversity within agricultural soil microbial communities. More extensive sampling is needed to better understand the driving forces underlying microbial community composition.


http://glycob.oupjournals.org/cgi/content/abstract/14/3/219

Galectins are a family of (beta)-galactoside-binding lectins that on synthesis are either translocated into the nucleus or released to the extracellular space. Their developmentally regulated expression, extracellular location, and affinity for extracellular components (such as laminin and fibronectin) suggest a role in embryonic development, but so far this has not been unequivocally established. Zebrafish constitute an ideal model for developmental studies because of their external fertilization, transparent embryos, rapid growth, and availability of a large collection of mutants. As a first step in addressing the biological roles in zebrafish embryogenesis, we identified and characterized members of the three galectin types: three protogalectins (Drgal1-L1, Drgal1-L2, Drgal1-L3), one chimera galectin (Drgal3), and one tandem-repeat galectin (Drgal9-L1). Like mammalian prototype galectin-1, Drgal1-L2 preferentially binds to N-acetyllactosamine. Genomic structure of Drgal1-L2 revealed four exons, with the exon-intron boundaries conserved with the mammalian galectin-1. Interestingly, this gene also encodes an alternatively spliced form of Drgal1-L2 that lacks eight amino acids near the
carbohydrate-binding domain. Zebrafish galectins exhibited distinct patterns of temporal expression during embryo development. Drgαl-L2 is expressed postbud stage, and its expression is strikingly specific to the notochord. In contrast, Drgαl-L1 is expressed maternally in the oocytes. Drgαl-L3, Drgαl3, and Drgαl9-L1 are expressed both maternally and zygotically, ubiquitously in the adult tissues. The distinct temporal and spatial patterns of expression of members of the zebrafish galectin repertoire suggest that each may play distinct biological roles during early embryogenesis.


We report here the purification, characterization, and cDNA cloning of a novel N-acetylgalactosamine-specific lectin from starfish, Asterina pectinifera. The purified lectin showed 19-kDa, 41-kDa, and 60-kDa protein bands on SDS-PAGE, possibly corresponding to a monomer, homodimer, and homotrimer. Interestingly, on 4-20% native PAGE the lectin showed at least nine protein bands, among which oligomers containing six to nine subunits had potent hemagglutination activity for sheep erythrocytes. The hemagglutination activity of the lectin was specifically inhibited by N-acetylgalactosamine, Tn antigen, and blood group A trisaccharide, but not by N-acetylgalactosamine, galactose, galactosamine, or blood group B trisaccharide. The specificity of the lectin was further examined using various glycosphingolipids and biotin-labeled lectin. The lectin was found to bind to Gb5Cer, but not Gb4Cer, Gb3Cer, GM1a, GM2, or asialo-GM2, indicating that the lectin specifically binds to the terminal (alpha)-GalNAc at the nonreducing end. The hemagglutination activity of the lectin was completely abolished by chelation with EDTA or EGTA and completely restored by the addition of CaCl2. cDNA cloning of the lectin showed that the protein is composed of 168 amino acids, including a signal sequence of 18 residues, and possesses the typical C-type lectin motif. These findings indicate that the protein is a C-type lectin. The recombinant lectin, produced in a soluble form by Escherichia coli, showed binding activity for asialomucin in the presence of Ca2+ but no hemagglutination.

Growth Hormone & IGF Research (2)


http://gut.bmjournals.com/cgi/content/abstract/53/4/501

Background: Stressful events in the early period of life (for example, maternal deprivation) have been shown to modify adult immune and gastrointestinal tract functions. The present study aimed to establish whether maternal deprivation affects colonic epithelial barrier and the development of an experimental colitis in adult rats. Methods: Male Wistar rat pups were separated during postnatal days 2-14 or left undisturbed with their dam. At 12 weeks of age, we assessed colonic paracellular permeability, bacterial translocation, myeloperoxidase (MPO) activity, mucosal mast cell density, cytokine (interleukin (IL)-1{beta}, IL-2, IL-4, IL-10, and interferon (gamma) (IFN-(gamma))) mRNA expression, and macroscopic damage. Total gut permeability, MPO activity, and macroscopic damage were also assessed four days after intracolonic administration of 2,4,6-trinitrobenzenesulphonic acid (TNBS). Results: Maternal deprivation triggered a significant increase in colonic permeability associated with bacterial translocation into the mesenteric lymph nodes, liver, and spleen. These alterations were associated with some macroscopic damage and an increase in colonic MPO activity, mucosal mast cell density, and cytokine mRNA expression. Intracolonic infusion of TNBS induced a significantly higher inflammatory reaction in separated animals, as judged by enhanced MPO colonic levels, total gut permeability, and macroscopic lesions. Conclusions: Maternal deprivation promotes long term alterations in the colonic epithelial barrier associated with an exaggerated immune response to an external immune stimulus. This suggests a role for early psychological factors in the regulation of colonic mucosal barrier in later life.


http://gut.bmjournals.com/cgi/content/abstract/53/12/1751

Background: Endogenous cyclooxygenase (COX) activity is required to maintain a relatively alkaline surface pH at the gastric luminal surface. Aims: The purpose of this study was to determine which COX isoform, COX-1 or COX-2, is responsible for regulating the protective surface pH gradient and to test if COX inhibitors also had non-COX mediated effects in vivo. Methods: Immunofluorescence and western blot analysis showed constitutive expression of both COX isoforms in the normal mouse stomach. We used in vivo confocal microscopy to measure pH near the mucosal surface of anaesthetised COX-1 (-/-), COX-2 (-/-), or wild-type mice of the same genetic background. Results: When the gastric mucosal surface was exposed and superfused (0.2 ml/min) with a weakly buffered saline solution (pH 3) containing the pH indicator CI-NERF, the pH directly at the gastric surface and thickness of the pH gradient were similar in wild-type and COX-2 (-/-) mice, but COX-1 (-/-) mice had a significantly thinner pH gradient. Addition of indomethacin had minimal effects on the residual surface pH gradient in COX-1 (-/-) mice, suggesting no role for COX-2 in surface pH regulation. Whole stomach perfusion studies demonstrated diminished net alkali secretion in COX-1 (-/-) mice, and application of SC-560 or rofecoxib to wild-type mice and mutant mice confirmed that only COX-1 inhibition reduced alkali
secretion. Conclusion: COX-1 is the dominant isoform regulating the normal thickness of the protective surface pH gradient in mouse stomach.


http://gut.bmjournals.com/cgi/content/abstract/51/5/677

Background: Germline mutations in the mismatch repair (MMR) genes hMLH1 and hMSH2 can cause hereditary non-polyposis colorectal cancer (HNPCC). However, the functional in vitro analysis of hMLH1 and hMSH2 mutations remains difficult. Aims: To establish an in vitro method for the functional characterisation of hMLH1 and hMSH2 mutations. Methods: hMLH1 and hMSH2 wild type (wt) genes and several mutated subclones were transiently transfected in mismatch repair deficient cell lines (HCT-116 and LOVO). Apoptosis, proliferation, and regulation of mRNA expression and protein expression of interacting proteins were analysed by Hoechst staining, AlamarBlue staining, real time polymerase chain reaction, and western blotting, respectively. Results: The protein expression of hMLH1 and hMSH2 mutants was significantly decreased after transfection compared with wild type transfections. The hMLH1 and hMSH2 interacting proteins hPMS2 and hMSH6 became detectable only after transfection of the respective wild type genes. In parallel, hMSH6 mRNA levels were increased in hMSH2 wt transfected cells. However, hPMS2 mRNA levels were independent of the mutation status of its interacting partner hMLH1, indicating a post-transcriptional regulating pathway. In the hMLH1 deficient HCT-116 cell line apoptosis was not affected by transfection of any mismatch repair gene, whereas complementation of hMSH2 deficiency in LOVO cells increased apoptosis. Conversely, proliferative activity of HCT-116 was decreased by complementation with hMLH1wt and unaffected in hMSH2 deficient LOVO cells. Conclusion: These data show that the cellular role of the MMR genes and its mutations are assessable in a simple transient transfection system and show the influence of MMR gene regulation on major cell growth regulating mechanisms. This method is applicable for the functional definition of mutations in hMLH1 and hMSH2 genes observed in patients with suspected HNPCC.


http://gut.bmjournals.com/cgi/content/abstract/53/12/1772

Background and aims: The cellular and molecular events involved in ischaemia reperfusion (IR) injury are complex and not fully understood. Previous studies have implicated polymorphonuclear neutrophils (PMN) as major inflammatory cells in IR injury. However, anti-PMN antiserum treatment offers only limited protection, indicating that other inflammatory cells are involved. We have therefore investigated the contribution of resident macrophages in IR injury using an IR gut injury model. Methods: DA rats were divided into sham operation and IR groups. The superior mesenteric artery was clamped for 30, 45, or 60 minutes (ischaemia) followed by 60 minutes of reperfusion. IR injuries were evaluated with histological staining. Expression of early growth response factor 1 (Egr-1), myeloperoxidase (MPO), and proinflammatory cytokines was analysed by immunohistochemistry, reverse transcription-polymerase chain reaction, and western blotting analysis. The specific role of macrophages in IR gut injury was also evaluated in resident macrophage depleted rats. Results: Mucosal sloughing and villi destruction were seen in 45/60 minute and 60/60 minute IR guts. PMN infiltration at the damaged mucosal area was undetectable in 45/60 minute and 60/60 minute IR guts. PMN were localised around the capillaries at the base of the crypts in 60/60 minute IR gut. Obviously PMN infiltration was only observed in damaged villi after three hours of reperfusion. Elevated nuclear Egr-1
immunostaining was localised in resident macrophages at the damaged villi before histological appearance of mucosal damage. Furthermore, resident macrophages at the damaged site expressed MPO. Protein levels of the proinflammatory cytokines RANTES and MCP-1 were increased in IR gut. Depletion of resident macrophages by dichloromethylene bisphosphonate significantly reduced mucosal damage in rat guts after IR. Conclusion: Our findings indicate that resident macrophages play a role in early mucosal damage in IR gut injury. Therefore, macrophages should be treated as a prime target for therapeutic intervention for IR damage.


http://gut.bmjjournals.com/cgi/content/abstract/53/5/710

Background: Several animal models for human ulcerative colitis (UC) associated neoplasia have been reported. However, most neoplasias developed in these models have morphological and genetic characteristics different from UC associated neoplasia. Aims: To establish a new colitis associated neoplasia model in p53 deficient mice by treatment with dextran sulphate sodium (DSS). Methods: DSS colitis was induced in homozygous p53 deficient mice (p53-/--DSS), heterozygous p53 deficient mice (p53+/--DSS) and wild-type mice (p53+/+-DSS) by treatment with 4% DSS. Numbers of developed neoplasias were compared among the experimental groups, and macroscopic and microscopic features of the neoplasias were analysed. Furthermore, K-ras mutation and beta-catenin expression were assessed. Results: p53-/--DSS mice showed 100% incidence of neoplasias whereas the incidences in p53+/--DSS and p53+/+-DSS mice were 46.2% and 13.3%, respectively. No neoplasias were observed in the control groups. The mean numbers of total neoplasias per mouse were 5.0 (p53-/--DSS), 0.62 (p53+/--DSS), and 0.2 (p53+/+-DSS). The number of neoplasias per mouse in the p53-/--DSS group was significantly higher than that in the other DSS groups. The incidences of superficial type neoplasias were 91.7% in p53-/--DSS mice, 75.0% in p53+/--DSS mice, and 33.3% in p53+/+-DSS mice. The K-ras mutation was not detected in any of the neoplasias tested. Translocation of beta-catenin from the cell membrane to the cytoplasm or nucleus was observed in 19 of 23 (82.6%) neoplasias. Conclusions: The p53-/--DSS mice is an excellent animal model of UC associated neoplasia because the morphological features and molecular genetics are similar to those of UC associated neoplasia. Therefore, this model will contribute to the analysis of tumorigenesis related to human UC associated neoplasia and the development of chemopreventive agents.


http://gut.bmjjournals.com/cgi/content/abstract/gut.2004.062059v1

Background: Surveillance colonoscopy is widely recommended in patients with long-standing and extensive ulcerative colitis (UC), in order to detect colorectal neoplasia at an early stage. However, it still remains questionable whether surveillance colonoscopy effectively enables early detection of UC-associated neoplasia. There is a great need for sensitive markers to identify individuals at increased risk of neoplasia. The estrogen receptor (ER) gene shows age-related methylation in the colorectal epithelium and is methylated frequently in sporadic colorectal neoplasia, suggesting that ER methylation might predispose to colorectal neoplasia. Aim: To clarify whether analysis of methylation of the ER gene in non-neoplastic epithelium can contribute to the prediction of increased neoplasia risk in UC patients. Materials and Methods: A total of 165 non- neoplastic colorectal epithelia from 30 patients with long-standing and extensive UC,
including 13 UC patients with neoplasia and 17 patients without, were evaluated. Methylation-specific PCR was performed to determine the methylation status of the ER gene. Results: Methylation of the ER gene was detected in 54 of 70 (77.1%) non-neoplastic colorectal epithelia in UC with neoplasia, whereas in only 23 of 95 (24.2%) without neoplasia. Methylation of the ER gene was significantly more frequent in non-neoplastic epithelium from UC with neoplasia than in chronic colitic epithelium from UC without neoplasia. In UC with neoplasia, furthermore, the ER gene was extensively methylated in non-neoplastic epithelia throughout the colorectum as compared with those in UC without neoplasia. Conclusion: These results suggest that analysis of ER gene methylation may have potential to be useful marker for identifying individuals at increased risk of neoplasia among patients with long-standing and extensive UC.


http://gut.bmjournals.com/cgi/content/abstract/52/4/563

Background: Hirschsprung's disease (HSCR) is a congenital disorder characterised by an absence of ganglion cells in the nerve plexuses of the lower digestive tract. Manifestation of the disease has been linked to mutations in genes that encode the crucial signals for the development of the enteric nervous system--the RET and EDNRB signalling pathways. The Phox2b gene is involved in neurogenesis and regulates Ret expression in mice, in which disruption of the Phox2b results in a HSCR-like phenotype. Aims: To investigate the contribution of PHOX2B to the HSCR phenotype. Methods: Using polymerase chain reaction amplification and direct sequencing, we screened PHOX2B coding regions and intron/exon boundaries for mutations and polymorphisms in 91 patients with HSCR and 71 ethnically matched controls. Seventy five HSCR patients with no RET mutations were independently considered. Haplotype and genotype frequencies were compared using the standard case control statistic. Results: Sequence analysis revealed three new polymorphisms: two novel single nucleotide polymorphisms (A→G1364; A→C2607) and a 15 base pair deletion (DEL2609). Statistically significant differences were found for A→G1364. Genotypes comprising allele G were underrepresented in patients (19% v 36%; \( \chi^2=9.30; p=0.0095 \) and 22% v 36%; \( \chi^2=7.38; p=0.024 \) for patients with no RET mutations). Pairwise linkage disequilibrium (LD) analysis revealed no LD between physically close polymorphisms indicating a hot spot for recombination in exon 3. Conclusion: The PHOX2B A→G1364 polymorphism is associated with HSCR. Whether it directly contributes to disease susceptibility or represents a marker for a locus in LD with PHOX2B needs further investigation. Our findings are in accordance with the involvement of PHOX2B in the signalling pathways governing the development of enteric neurones.


http://gut.bmjournals.com/cgi/content/abstract/52/1/47

Background: It is well established that the wheat protein gliadin triggers inflammation in coeliac patients. However, the potential toxicity of avenin, the equivalent protein in oats, is debated. Aim: To investigate the immunogenicity of avenin using the cytokines interferon (\( \gamma \)) (IFN-\( \gamma \)) and interleukin (IL)-2 as markers of immunological activity. Methods: Duodenal biopsies from coeliac patients were cultured with 5 mg/ml of peptic tryptic (PT) gliadin (n=9) or 5 mg/ml of PT avenin (n=8) for four hours. Biopsies cultured with RPMI 1640 alone served as controls. Non-coeliac biopsies were also cultured with PT gliadin (n=8) and PT avenin (n=8). Total RNA was extracted from the tissue after culture. Cytokine mRNA was quantified by TaqMan
polymerase chain reaction. Secreted cytokine protein was measured in the culture supernatant by enzyme linked immunosorbent assay. Results: After culture with PT gliadin, an increase in IFN-{gamma} mRNA was observed in all nine patients with coeliac disease. Increased IFN-{gamma} protein was also found in four of these patients. Smaller increases in IL-2 mRNA were detected in six subjects with increased IL-2 protein found in two patients. In contrast with PT gliadin, there was no significant IFN-{gamma} or IL-2 response when coeliac biopsies were cultured with PT avenin. Similarly, biopsies from normal controls did not respond to PT gliadin or PT avenin stimulation. Conclusions: The findings of this study suggest that the immunogenic sequences in gliadin are not present in avenin. Moreover, they are in keeping with in vivo studies which report that oats are safe for consumption by coeliac patients.


http://gut.bmjournals.com/cgi/content/abstract/52/8/1148

Background and aims: Colorectal epithelial cells are prone to malignant transformation. Therefore, identification of differences in gene expression in the process from normal colonic crypts to adenomas with low grade dysplasia is essential for further insights into early tumorigenesis. To achieve this goal, a novel gene expression analysis strategy, screening for expressed transcripts in small histologically defined tissue samples, was performed. Methods: First, laser mediated microdissection was used to isolate normal and adenomatous crypts from colonic cryosections. Then, nested RNA arbitrarily primed polymerase chain reaction (RAP-PCR) for differential display was performed to screen mRNA populations and to generate hybridisation probes for cDNA expression arrays. After evaluation of cDNA expression arrays, differential expression was confirmed at the protein level by immunohistochemistry. Results: Evaluation of gene expression profiles of normal versus adenomatous colonic crypts of six different patients revealed, in general, dysregulation of up to 11% of all analysed genes (total number n=588): specifically, p21-rac1 was upregulated in four of six patients, mitogen activated protein kinase (MAPK) p38(alpha) in three of six patients, and interferon (gamma) receptor in three of six patients. Conversely, FAST kinase was found to be downregulated in three of six patients, p53 in three of six patients, and thrombospondin 2 in three of six patients. Conclusions: For the first time, distinct gene expression profiles of dysplastic areas within colonic adenomas, using the combination of laser mediated microdissection with RAP-PCR and cDNA expression array, were shown. In these samples, upregulation of proliferation associated genes (ras-oncogene related p21-rac1 and MAPK p38(alpha)) as well as downregulation of apoptosis related genes (FAST kinase and p53) most likely reflects specific alterations in adenomas with low grade dysplasia. Based on upregulation of p21-rac1 and MAPK p38(alpha), activation of the MAPK pathway appears to be an early event in colonic carcinogenesis.


http://gut.bmjournals.com/cgi/content/abstract/53/3/331

Background and aims: Although peroxisome proliferator activated receptor (gamma) (PPAR(gamma)) agonists have been implicated in differentiation and growth inhibition of cancer cells, the potential therapeutic and chemopreventive effects on gastric cancer are poorly defined. We examined the in vitro and in vivo effects of PPAR(gamma) ligands on growth of gastric cancer, and the effect of PPAR(gamma) activation on expression of cyclooxygenase 2 (COX-2) and cancer related genes. Methods: Gastric cell lines (MKN28 and MKN45) were treated with two specific PPAR(gamma) ligands: ciglitazone and 15-deoxy-[Delta]12,14-prostaglandin J2. Cell
growth was determined by bromodeoxyuridine incorporation assay and apoptosis was measured by DNA fragmentation. Expression of COX-2 was determined by western blot and real time quantitative polymerase chain reaction (PCR). Expression profiles of cancer related genes were screened with cDNA array. In vivo growth of implanted MKN45 cells in nude mice was monitored after oral treatment with rosiglitazone. Results: PPAR(\gamma) ligands suppressed the in vitro growth of MKN45 cells in a dose dependent manner whereas prostacyclin, a PPAR(\delta) agonist, had no growth inhibitory effect. Growth inhibition was more pronounced in MKN45 cells, which was accompanied by DNA fragmentation and downregulation of COX-2. Screening by cDNA microarray showed that PPAR(\gamma) ligand treatment was associated with upregulation of bad and p53, and downregulation of bcl-2, bcl-xl, and cyclin E1 in MKN45 cells, which was confirmed by quantitative real time PCR. In contrast, MKN28 cells with lower PPAR(\gamma) and COX-2 expression levels had lower growth inhibitory responses to PPAR(\gamma) ligands. Microarray experiments only showed induction of the bad gene in MKN28 cells. In vivo growth of MKN45 cells in nude mice was retarded by rosiglitazone. Mean tumour volume in rosiglitazone treated mice was significantly lower than controls at six weeks (p = 0.019) and seven weeks (p = 0.001) after treatment. Conclusions: PPAR(\gamma) ligands suppress both in vitro and in vivo growth of gastric cancer and may play a major role in cancer therapy and prevention.


http://gut.bmjournals.com/cgi/content/abstract/52/4/541

Background and aims: Genetic variation in the chromosome 5q31 cytokine cluster (IBD5 risk haplotype) has been associated with Crohn's disease (CD) in a Canadian population. We studied the IBD5 risk haplotype in both British and Japanese cohorts. Disease associations have also been reported for CARD15/NOD2 and TNF variants. Complex interactions between susceptibility loci have been shown in animal models, and we tested for potential gene-gene interactions between the three CD associated loci. Methods: Family based association analyses were performed in 457 British families (252 ulcerative colitis, 282 CD trios) genotyped for the IBD5 haplotype, common CARD15, and TNF-857 variants. To test for possible epistatic interactions between variants, transmission disequilibrium test analyses were further stratified by genotype at other loci, and novel log linear analyses were performed using the haplotype relative risk model. Case control association analyses were performed in 178 Japanese CD patients and 156 healthy controls genotyped for the IBD5 haplotype. Results: The IBD5 haplotype was associated with CD (p=0.007), but not with UC, in the British Caucasian population. The CARD15 variants and IBD5 haplotype showed additive main effects, and in particular no evidence for epistatic interactions was found. Variants from the IBD5 haplotype were extremely rare in the Japanese. Conclusions: The IBD5 risk haplotype is associated with British CD. Genetic variants predisposing to CD show heterogeneity and population specific differences.


http://gut.bmjournals.com/cgi/content/abstract/53/5/685

Background and aims: The intestinal bacterial microflora plays an important role in the aetiology of inflammatory bowel disease (IBD). As most of the colonic bacteria cannot be identified by culture techniques, genomic technology can be used for analysis of the composition of the microflora. Patients and methods: The mucosa associated colonic microflora of 57 patients with active inflammatory bowel disease and 46 controls was investigated using 16S rDNA based single strand conformation polymorphism (SSCP) fingerprint, cloning experiments, and real time
polymerase chain reaction (PCR). Results: Full length sequencing of 1019 clones from 16S rDNA libraries \((n = 3)\) revealed an overall bacterial diversity of 83 non-redundant sequences—among them, only 49 known bacterial species. Molecular epidemiology of the composition of the colonic microflora was investigated by SSCP. Diversity of the microflora in Crohn's disease was reduced to 50% compared with controls \((21.7 \pm 50.4; p<0.0001)\) and to 30% in ulcerative colitis \((17.2 \pm 50.4; p<0.0001)\). The reduction in diversity in inflammatory bowel disease was due to loss of normal anaerobic bacteria such as Bacteroides species, Eubacterium species, and Lactobacillus species, as revealed by direct sequencing of variable bands and confirmed by real time PCR. Bacterial diversity in the Crohn's group showed no association with CARD15/NOD2 status. Conclusions: Mucosal inflammation in inflammatory bowel disease is associated with loss of normal anaerobic bacteria. This effect is independent of NOD2/CARD15 status of patients.


http://gut.bmjjournals.com/cgi/content/abstract/52/2/231

Background: Superoxide \((O_2^-)\) generation through the activity of reduced nicotinamide dinucleotide \((NADH)\) or reduced nicotinamide dinucleotide phosphate \((NADPH)\) oxidases has been demonstrated in a variety of cell types, but not in human colonic epithelial cells. Aims: To measure \(O_2^-\) production and effects of modulators of NAD(P)H oxidase activity and inhibitors of potential \(O_2^-\) generating enzymes in cultures of human colonic epithelial cells. Expression of the catalytic subunits of NAD(P)H oxidase, Nox1 and gp91phox (phox, phagocytic oxidase), and the membrane bound subunit p22phox was assessed. Methods: The transformed colonic epithelial cell lines (DLD-1, HT-29, and Caco-2) were studied at subconfluence, confluence, and after differentiation. Primary colonic epithelial cells were isolated from mucosal biopsies from the normal human colon. Extracellular \(O_2^-\) production was measured by the cytochrome c reduction assay or luminol enhanced luminescence. Nox1, gp91phox, and p22phox mRNA expression was assessed in colonic epithelial cells and blood neutrophils by reverse transcriptase-polymerase chain reaction. Results: Production rates of \(O_2^-\) were higher in subconfluent transformed cells \((\text{mean (SEM)} 35.8 (4.2) \text{nmol/mg of protein/h})\) and primary cells \((40.4 (5.9)\) than in confluent transformed cells \((6.0 (0.9); p<0.01)\). The oxidoreductase inhibitor diphenylene iodonium significantly inhibited \(O_2^-\) production whereas NADPH and NADH increased production rates. In contrast, \(O_2^-\) was unaffected by phorbol myristate ester, NG-nitro-L-arginine methyl ester, indomethacin, or allopurinol. Nox1 mRNA was expressed in all colonic epithelial cells whereas gp91phox was detected only in HT-29 cells and neutrophils. p22phox was expressed in all cell types. Conclusions: Cultures of transformed and primary epithelial cells from human colon may produce extracellular \(O_2^-\) through an NAD(P)H oxidase expressing Nox1 and p22phox.


http://gut.bmjjournals.com/cgi/content/abstract/52/7/1060

Background and aim: Liver regeneration after severe liver damage depends in part on proliferation and differentiation of hepatic progenitor cells (HPCs). Under these conditions they must be able to withstand the toxic milieu of the damaged liver. ATP binding cassette (ABC) transporters are cytoprotective efflux pumps that may contribute to the preservation of these cells. The aim of this study was to determine the ABC transporter phenotype of HPCs. Methods: HPC activation was studied in rats treated with 2- acetylaminofluorene \((2-\text{AAF})\) followed by partial hepatectomy \((\text{PHx})\). ABC transporter gene expression was determined by real time detection reverse transcription-polymerase chain reaction in isolated HPCs, hepatocytes, cholangiocytes,
and cultured progenitor cell-like RLF \{\phi\} 13 cells and by immunohistochemistry of total liver samples. ABC transporter efflux activity was studied in RLF \{\phi\} 13 cells by flow cytometry.

Results: 2-AAF/PHx treated animals showed increased hepatic mRNA levels of the genes encoding multidrug resistance proteins Mdr1b, Mrp1, and Mrp3. Immunohistochemistry demonstrated expression of Mrp1 and Mrp3 proteins in periportal progenitor cells and of the Mdr1b protein in periportal hepatocytes. Freshly isolated Thy-1 positive cells and cultured RLF \{\phi\} 13 progenitor cells highly expressed Mrp1 and Mrp3 mRNA while the hepatocyte specific transporters Mdr2, Bsep, Mrp2, and Mrp6 were only minimally expressed. Blocking Mrp activity by MK-571 resulted in accumulation of the Mrp specific substrate carboxyfluorescein in RLF \{\phi\} 13 cells. Conclusion: HPCs express high levels of active Mrp1 and Mrp3. These may have a cytoprotective role in conditions of severe hepatotoxicity.


http://gut.bmjournals.com/cgi/content/abstract/53/7/1001

Background and aim: Significant telomere shortening of hepatocytes is associated with replicative senescence and a non-dividing state in chronic liver disease, resulting in end stage liver failure and/or development of hepatocellular carcinoma. To prevent critical telomere shortening in hepatocytes, we have focused on oestrogen dependent transactivation of the human telomerase reverse transcriptase (hTERT) gene as a form of telomerase therapy in chronic liver disease.

Methods: We examined expression of hTERT mRNA and its protein, and telomerase activity (TA) in three human normal hepatic cell lines (Hc-cells, h-Nheps, and WRL-68) before and after treatment with 17\{beta\}-oestradiol. The effects of exogenous oestradiol administration were examined in a carbon tetrachloride (CCI4) induced model of liver fibrosis in rats. Results: Expression of hTERT mRNA and its protein was upregulated by oestradiol treatment. Telomere length decreased in Hc-cells and h-Nheps with accumulated passages whereas with long term oestradiol exposure it was greater than without oestradiol. The incidence of \{beta\}-galactosidase positive cells, indicating a state of senescence, decreased significantly in oestradiol treated cells in comparison with non-treated cells (p<0.05). TA in both male and female rats with CCI4 induced liver fibrosis was significantly higher with oestradiol administration than without (p<0.05). Long term oestradiol administration markedly rescued the hepatic telomere from extensive shortening in both male and female rats. Conclusion: These results suggest that oestradiol acts as a positive modulator of the hTERT gene in the liver. Oestrogen dependent transactivation of the hTERT gene is a new strategy for slowing the progression of chronic liver disease.


http://gut.bmjournals.com/cgi/content/abstract/50/4/530

Background: Although circulating tumour DNA has been detected in patients with different types of cancer, little is known of free RNA in cancer patients. Aims: We investigated the presence of RNA from epithelial tumours in plasma from patients with colorectal carcinomas, and its correlation with tumour characteristics and circulating tumour cells. Methods: \{beta\}-actin mRNA was analysed to assess the viability of plasma RNA in samples from 53 patients with colonic cancer and 25 controls. Subsequently, nested primers were used to detect the presence of cytokeratin 19 (CK19) and carcinoembryonic antigen (CEA) RNA in the same samples. Nine clinicopathological parameters were studied to correlate the molecular and clinical parameters. Additionally, we investigated for micrometastases in blood in 18 of these patients and in 10 of the
controls samples. Results: All samples had detectable quantities of (beta)-actin RNA. In the controls, one case (4%) was positive for CEA and five (20%) for CK19 RNA; of the 53 patients, 17 cases (32%) were positive for CEA and 39 (73.6%) for CK19 RNA. This was statistically significant (p=0.000001). Advanced stages (p=0.03) and soluble CEA status (p=0.03) were associated with the presence of CEA, CK19, or both RNAs in plasma. Lymph node metastases (p=0.06) and vascular invasion (p=0.07) were almost significant. On the basis of these results, we examined the possible presence of micrometastases in blood in several of these patients. The presence of plasma tumour RNA was found to be associated with circulating tumour cells in blood (p=0.04). Conclusions: Epithelial tumour RNA is detectable in plasma from colon cancer patients. This molecular event is associated with advanced stages and circulating tumour cells. Our results could offer new approaches in the diagnosis and monitoring of colon cancer.


http://gut.bmjournals.com/cgi/content/abstract/52/5/706

Backgrounds: The Raf/MEK/ERK (mitogen activated protein kinase--MAPK) signal transduction cascade is an important mediator of a number of cellular fates, including growth, proliferation, and survival. The BRAF gene, one of the human isoforms of RAF, is activated by oncogenic Ras, leading to cooperative effects in cells responding to growth factor signals. Aims: The aim of this study was to elucidate a possible function of BRAF in liver tumours. Methods: Mutations of BRAF and KRAS were evaluated in 25 hepatocellular carcinomas (HCC) and in 69 cholangiocarcinomas (CC) by direct DNA sequencing analyses after microdissection. The presence of active intermediates of the MAPK pathway was assessed immunohistochemically. The results obtained were correlated with histopathological variables and patient survival. Results: Activating BRAF missense mutations were identified in 15/69 CC (22%) and in one case of tumour surrounding liver. KRAS mutations were found in 31 of 69 (45%) CC examined and in two cases of tumour surrounding non-neoplastic liver tissue. In HCC, neither BRAF nor KRAS mutations were detected. All 31 CC with KRAS mutations had an intact BRAF gene. We failed to observe a correlation between BRAF or KRAS mutations and histopathological factors or prognosis of patients. Conclusions: Our data indicate that BRAF gene mutations are a relatively common event in CC but not in HCC. Disruption of the Raf/MEK/ERK (MAPK) kinase pathway, either by RAS or BRAF mutation, was detected in approximately 62% of all CC and is therefore one of the most frequent defects in cholangiocellular carcinogenesis.

Waidmann, M., Y. Allemand, et al. (2002). "Microflora reactive IL-10 producing regulatory T cells are present in the colon of IL-2 deficient mice but lack efficacious inhibition of IFN-(gamma) and TNF-(alpha) production." Gut 50(2): 170-179.

http://gut.bmjournals.com/cgi/content/abstract/50/2/170

Background: Inflammatory bowel disease in interleukin 2 (IL-2) deficient (IL-2/-/-) mice is triggered by the intestinal microflora and mediated by CD4+ T cells. Aims: To determine the characteristics of microflora specific intestinal T cells, including migration and cytokine production. Methods: Intestinal T cell populations and cytokine mRNA expression of specific pathogen free (SPF) and germ free (GF) IL-2/-/- and IL-2/+/+ mice were compared by flow cytometry and reverse transcription-polymerase chain reaction. Cytokine production of intestinal mononuclear cells on stimulation with microflora antigens was assessed by ELISA. In vivo migration of T cells was assessed by adoptive transfer of 51Cr labelled CD4+CD25-(alpha)(beta)+ T cells. The ability of intestinal T cell lines to promote colitis was determined by adoptive transfer experiments. Results: SPF IL-2/-/- mice produced higher interferon (gamma) (IFN-(gamma)) and tumour necrosis factor
mRNA levels than GF IL-2-/ mice, which was accompanied by an increased number of CD4+CD8 T cells in the colon. Tracking of 51Cr labelled and adoptively transferred T cells revealed an increased MadCAM-1 dependent but VCAM-1 independent recruitment of these cells into the colon of SPF IL-2-/ mice. Colon lamina propria lymphocytes (LPL) from SPF IL-2-/ mice showed increased spontaneous IFN-γ production in vitro. On stimulation with bacterial microflora antigens, intraepithelial lymphocytes and LPL did not produce IFN-γ, but high quantities of IL-10, which did not suppress IFN-γ production. Bacterial antigen specific cell lines established from colon LPL of SPF IL-2-/ mice with colitis showed a regulatory T cell-like cytokine profile and only marginally modulated the course of colitis and survival of IL-2-/ mice. Conclusions: Our results suggest that microflora reactive regulatory T cells are present in the colon of SPF IL-2-/ mice. However, IL-10 produced by these cells did not significantly modulate a possible secondary proinflammatory CD4 Th1 cell population to produce IFN-γ.


http://gut.bmjournals.com/cgi/content/abstract/53/10/1452

Background and aims: Serotonin (5-hydroxytryptamine, 5-HT) is an important factor in gut function, playing key roles in intestinal peristalsis and secretion, and in sensory signalling in the brain-gut axis. Removal from its sites of action is mediated by a specific protein called the serotonin reuptake transporter (SERT or 5-HTT). Polymorphisms in the promoter region of the SERT gene have effects on transcriptional activity, resulting in altered 5-HT reuptake efficiency. It has been speculated that such functional polymorphisms may underlie disturbance in gut function in individuals suffering with disorders such as irritable bowel syndrome (IBS). The aim of this study was to assess the potential association between SERT polymorphisms and the diarrhoea predominant IBS (dIBS) phenotype. Subjects: A total of 194 North American Caucasian female dIBS patients and 448 female Caucasian controls were subjected to genotyping. Methods: Leucocyte DNA of all subjects was analysed by polymerase chain reaction based technologies for nine SERT polymorphisms, including the insertion/deletion polymorphism in the promoter (SERT-P) and the variable tandem repeat in intron 2. Statistical analysis was performed to assess association of any SERT polymorphism allele with the dIBS phenotype. Results: A strong genotypic association was observed between the SERT-P deletion/deletion genotype and the dIBS phenotype (p = 3.07x10-5; n = 194). None of the other polymorphisms analysed was significantly associated with the presence of disease. Conclusions: Significant association was observed between dIBS and the SERT-P deletion/deletion genotype, suggesting that the serotonin transporter is a potential candidate gene for dIBS in women.


http://gut.bmjournals.com/cgi/content/abstract/51/4/480

Background: Helicobacter pylori blood group antigen binding adhesin (BabA) mediates bacterial adherence to human blood group antigens on gastric epithelium. Although strains harbouring babA2 were recently found to be associated with peptic ulcer and gastric cancer, the role of babA2 in cellular turnover, severity of gastritis, and premalignant changes is poorly understood. Aim: We correlated H pylori babA2, vacuolating toxin (vacA), and cytoxin associated gene A (cagA) genotypes with the severity of gastric inflammation and epithelial cell turnover in a group of Chinese patients from an area with a high incidence of gastric cancer. Patients and methods: H
Pylori isolates were obtained from 104 Chinese patients who participated in a gastric cancer prevention programme. Genotype variants of babA2, vacA, and cagA were determined by polymerase chain reaction. Antrum and corpus histopathology was examined according to the updated Sydney classification. Apoptosis was scored by terminal uridine deoxynucleotidyl nick end labeling (TUNEL) and proliferation by Ki-67 immunostaining. Results: Of the 104 patients, 102 (98.1%) harboured cagA+ strains and all had vacA s1 genotype. The babA2+ strains were found in 83 (79.8%) patients and were associated with higher lymphocytic infiltration (p=0.028), presence of glandular atrophy (odds ratio (OR) 7.5, 95% confidence interval (CI) 2.3-24.3), and intestinal metaplasia (OR 7.4, 95% CI 2.2-25.3) in the antrum. Increased epithelial proliferation was also noted in individuals infected with babA2+ strains (p=0.025). Strains harbouring cagA+/vacA s1 genotypes lacked this association in the absence of babA2. Conclusions: The presence of babA2+ H pylori strains alone or in combination with cagA+ and vacA s1 was associated with the presence of preneoplastic gastric lesions.

Gynecologic Oncology

http://www.sciencedirect.com/science/article/B6WG6-4FPYWD- C/2/49bd69686d85ac8e5b00adde5ba2a73f

Objective(s)To compare the type-specific human papillomavirus (HPV) recovery from physician and patient-collected samples.MethodsThree hundred thirty-four (334) women attending colposcopy clinics in three countries were enrolled in this cross-sectional study. Cervicovaginal samples were collected by patients and physicians and processed with polymerase chain reaction and reverse line blot genotyping. McNemar's Chi-squared tests and Kappa statistics were utilized to determine statistical associations between physician- versus patient-collected samples.ResultsOncogenic HPV infection was identified in 23.2% of patient-collected specimens compared to 34.9% of physician-collected specimens. Physician sampling detected significantly more infections with type 16 and 52 than did self-sampling and significantly more oncogenic HPV infection overall. For non-oncogenic HPV detection, there was no statistical difference between physician- and patient-collected samples.Conclusion(s)Patient sampling for HPV using a single vaginal brush does not identify all oncogenic HPV subtypes.

http://www.sciencedirect.com/science/article/B6WG6-4840PC0- 9/2/8f0a6a22447cdaaa988e810fe9ea615c

ObjectiveOsteopontin (OPN) is a glycoprotein of the extracellular matrix that can bind to different types of receptors including integrins and CD44 receptors. Multiple binding affinity enables OPN to play a role in many physiological and pathological processes. OPN contributes to tumorigenesis in several types of cancers. OPN is also expressed by the endometrium and by trophoblast cells of the chorionic villus in human placenta, where OPN may regulate implantation and placentation in early pregnancies by promoting cell-cell interactions, adhesion, spreading, and migration of trophoblast. Our purpose was to determine the expression of OPN mRNA and
protein in hydatidiform mole and in normal placenta of comparable gestational age. Methods A total of 13 fresh tissues from complete hydatidiform moles, 2 from partial hydatidiform moles, and 9 from normal placentas were analyzed by performing quantitative real-time PCR on microdissected trophoblast cells and immunohistochemistry on frozen sections of tissue. Results Our results showed significantly lower expression of OPN mRNA and protein in hydatidiform mole, and in particular complete mole (P = 0.001 by real-time PCR and P Conclusion Although precise molecular mechanisms of gestational trophoblastic diseases have not yet been determined, down-regulation of osteopontin may play an important role in the pathogenesis of molar pregnancy.


http://www.sciencedirect.com/science/article/B6WG6-4D9RNJ-3/2/2c5ef673603d9aa13111f3dccc71a1

Objectives A deletion variant in the CHEK2 gene (del1100C) has been implicated as a low-penetrance risk factor for breast cancer. We sought to determine contribution of CHEK2 mutations to the etiology of ovarian cancer (OvCa). Methods We used cases ascertained from the United States through Gynecologic Oncology Group (GOG) protocols 172, 182, and 144, the University of Hawaii Cancer Research Center, and Creighton University. Control women were recruited from Pittsburgh and Hawaii. Denaturing high-performance liquid chromatography, sequence analysis, and single nucleotide polymorphism genotyping by Pyrosequencing were employed to analyze the CHEK2 gene. Results Mutation screening of the CHEK2 gene in 48 cases who had a first-degree relative with OvCa uncovered only del1100C and A252G variants. Altogether, the del1100C variant was detected in none of 751 unselected cases, in 1 of 52 (1.9%) cases who had a first-degree relative with OvCa, and in 3 of 521 (0.6%) unselected controls. The frequencies of del1100C and A252G variants did not show statistically significant differences between the cases and the controls. Conclusions These results suggest that variations in CHEK2 do not make a significant contribution to the pathogenesis of OvCa in the U.S. population.


http://www.sciencedirect.com/science/article/B6WG6-4C5HR81-4/2/e367592766f9b89c1a20d680c942e5b0

Objectives. We studied the role of epigenetic and genetic changes of PTEN in the development of squamous cell carcinoma (SCC) of the uterine cervix and their value as a prognostic factor. Methods. Ten high-grade cervical intraepithelial neoplasia (CIN-H) and 62 SCC tissues were used in this study. Microdissection was performed before loss of PTEN function through methylation of promoter CpG islands, deletion and mutation were studied. The findings were verified with PTEN protein expression and correlated with clinicopathologic information. Results. PTEN mutation assessed by single-strand conformation polymorphism (PCR-SSCP) was not noted in any of the 62 SCC. Loss of heterozygosity (LOH) was only seen in eight SCC. PTEN promoter methylation was detected in 40% (4/10) of CIN-H and 58% (36/62) of SCC specimens. Loss of PTEN protein expression was associated with methylation of PTEN. PTEN methylation was not related to patient age, tumor grade or stage. Patients with persistent disease or who died of disease had a significantly higher percentage of PTEN methylation than those without evidence of recurrence. Multivariate Cox regression models confirmed PTEN was an important significant predictor both for total and disease-free survival after controlling age, pathologic grade and clinical stage. Conclusions. PTEN methylation and loss of PTEN expression are early events
in the development of cervical cancer and may have prognostic significance.


http://www.sciencedirect.com/science/article/B6WG6-4F05G1K-Fiz/4aa71d09ecd5602c57b6fe025e05cb0

Objectives We studied the loss of heterozygosity (LOH) in chromosome 1 in squamous cell carcinoma (SCC) of the uterine cervix and evaluated its clinical and pathological significance. Methods Sixty-three highly polymorphic markers were used to study the LOH in 84 SCC. Microdissection was performed to enrich the tumor cells population before the alleotyping study. The findings were correlated with clinicopathologic findings. Results LOH was detected in all but one SCC. The number of loci showing LOH in each case ranged from 0 to 41. Five loci showed LOH in ≥30% SCC and 28 other loci had an LOH frequency between 20% and 30%. Six of the eight markers located at 1p36.21 to 1p36.33 had a frequency of LOH >20%. Shortened total survival was associated with LOH at 14 loci and shortened disease-free survival was associated with LOH at 11 loci while LOH at nine loci were associated with both. A high frequency of LOH was associated with stage as well as shortened total and disease-free survival. Conclusions LOH is a common and early event in the development of cervical SCC. Tumor suppressor genes may be present at 1p36. The incidence of LOH increases as the tumor progresses but a high frequency of LOH is not an independent prognostic factor.


http://www.sciencedirect.com/science/article/B6WG6-48N2PVH-9/2/b7d6a509fb3a4b5d678ed702b9fa1302

Introduction We aimed to verify not only whether homozygous Arg at codon 72 of the p53 apoptotic domain is a possible risk factor for cervical human papillomavirus (HPV)-related cancer, but whether degraded p53 may have an effect on a G2 checkpoint of the cell cycle. The implication of the codon 72 polymorphism of p53 in cervical tumor remains controversial. Furthermore, G2 checkpoint alteration and its relationship with p53, the codon 72 alletype, according to HPV infection in cervical tumors, has not been studied. Materials and methods The purified genomic DNA from 252 archival cervical tissues [102 cervical intraepithelial neoplasias (CINs) and 46 squamous cell carcinomas of the uterine cervix (SCCs), and 104 normal] were amplified by nested polymerase chain reaction (PCR) for HPV-16/HPV-18. In addition, all of them were amplified by PCR for exon 4 of p53, where the codon 72 resides. The amplified PCR products were then sequenced using the forward primer. A polymorphism analysis was done by Snapshot ddNTP primer extension and following direct sequencing. The reaction mixture was treated with 0.25 unit of shrimp alkaline phosphatase (Amersham) at 37[deg]C for 1 h, subsequently performed in an ABI Prism 310 Genetic Analyzer (Perkin-Elmer). The archival slides were incubated overnight at 4[deg]C using mouse anti-human recombinant cyclin B1 polyclonal antibody or mouse anti-Xenopus p34cdc2 monoclonal antibody for immunohistochemistry (Santa Cruz Biotech, Santa Cruz, CA). Results The frequency of Arg allelic homozygosity was high in both cases (89.1%) and the control (80.8%) group (P = 0.4703). All groups except CIN were in Hardy-Weinberg equilibrium. There was no significant difference in the frequency of p53 polymorphism between the HPV-positive (Arg, 88.0%) and the negative (Arg, 88.8%) groups, or between CIN (Arg, 88.2%) and SCC (Arg, 89.1%). Both immunoreactivities to cyclin B and p34cdc2 were strongly correlated with the HPV infection (P = 0.0001) and the histological types (P = 0.0001) between CIN and SCC, being strongly correlated with each other.
The particular type of the p53 polymorphism does not bear relation to the progression of cervical cancer, HPV infection, or to the p53 codon 72 polymorphism. However, the G2 checkpoint appears to be altered in the case of a HPV-positive SCC.


Objectives. Microarray expression analysis of cervical tumors has revealed differential expression of genes that may be useful as markers or targets for treatment. We question the application of array findings across the major categories of cervical cancer. We sought to identify differences between normal squamous epithelium (NSQ) and glandular epithelium (NGL) of the uterine cervix and their malignant variants: squamous cell cancer (SCC) and adenocarcinoma (ACA).

Methods. Eight genes were selected: 12-lipoxygenase (12-LOX), keratin 4, trypsinogen 2 (TRY2), Rh glycoprotein C (RhGC), collagen type V alpha 2, integrin alpha 5, integrin alpha 6, and c-myc. Ten cases each of SCC and ACA of the cervix were selected from our tumor bank. NSQ and NGL epithelia were obtained from consecutive patients undergoing surgery for benign disease. RNA extraction, cDNA synthesis, and DNA amplification of all samples were performed according to an established protocol. Electrophoresis of the multiplex polymerase chain reaction (PCR) products was performed under standard conditions, followed by digital image capture. A ratio of target to control gene (β-actin) was obtained for each sample. Analysis of variance was applied to the mean ratios for each tissue to establish significant differences. Individual pairwise comparisons were made by Student t-tests and verified with the Tukey-Kramer test.

Results. Clinically valid comparisons are NSQ to NGL, NSQ to SCC, NGL to ACA, and SCC to ACA. Various expression patterns were observed between the epithelia and their malignant phenotypes. Significant differences in gene expression were observed between benign squamous and glandular epithelium in four of the eight genes and between malignant squamous and glandular epithelium in three of the eight genes. Significant differences in gene expression between benign and malignant tissues were demonstrated in four of the eight genes.

Conclusions. We have defined significant differential expression changes between the two principal cervical tumor types. Differences in genes are demonstrated and must be considered if array technology is applied to the study of the biologic behavior of these tumors as well as their screening and management. The observed differential expression should be a compelling argument to perform type-specific expression analysis for other tumors with histological variants.


Background. Cervical cancer represents a major health problem in Venezuela as well as in other Latin American countries. High-risk human papillomavirus (HR-HPV) infection is known as the major risk factor of cervical cancer. However, whether or not a HR-HPV-infected woman progresses to cervical cancer may depend on the immune system effectors induced by viral antigens presented by her specific human leukocyte antigens (HLA) alleles. The role of the HLA system in presenting peptides to antigen-specific T-cells may be critical for genetic susceptibility and genetic resistance to cervical carcinoma.

Objective. We aimed to investigate the relationship between HLA-DQB1, HPV infection, and cervical cancer in Venezuelan women.

Methods. Blood
samples and cervical swabs were obtained from 36 patients and 79 healthy controls; additional
 cervical biopsies were obtained from all the patients. HPV DNA was detected by PCR and HLA-
DQB1 genotyping was performed using a PCR-SSP protocol.

Results. A positive association with cervical cancer was observed for HLA-DQB1*0201-0202 and *0402 alleles, however after
Bonferroni correction only HLA-DQB1*0402 remained statistically significant (P value = 0.004, RR
= 5.067).

Conclusion. This is the first report of HLA-DQB1 alleles associated with cervical
 carcinoma in Venezuelan women. Larger studies are needed to assess whether these HLA-
DQB1*0201-0202 and *0402 alleles have a direct effect on disease susceptibility.

Dowdy, S. C., A. Mariani, et al. (2005). "Overexpression of the TGF-[beta] antagonist Smad7 in
dermatial cancer." Gynecologic Oncology 96(2): 368.

http://www.sciencedirect.com/science/article/B6WG6-4DTSJH7-
7/2/4f43c33eada14822b9a699cb2c900df7

Objective. We have shown that HER2/Neu may activate the Smad7 promoter in endometrial,
ovarian, and breast cancer cell lines. Elevated Smad7 levels could then antagonize the TGF-
[beta] pathway, leading to a reduction in tumor surveillance and potential cancer formation. Our
aim was to determine if Smad7 was in fact overexpressed in endometrial cancers and whether
Smad7 RNA levels correlated with tumor grade or clinical endpoints.

Methods. Snap-frozen endometrial cancer specimens from 16 patients with grade 1 disease and 23 patients with grade
3 disease were obtained. Additionally, the endometrium from 18 patients who underwent
hysterectomy for benign indications was collected as a control. RNA was extracted and subjected
to quantitative real-time PCR to determine the degree of Smad7 RNA expression. Clinical
outcomes including time to recurrence were recorded through retrospective chart
review.

Results. Smad7 transcripts in the tumors were over 11-fold elevated on average than in
controls (P Smad7 RNA between grades 1 and 3 tumors. For the 19 patients who recurred,
median time to recurrence was 56.3 months for those with low Smad7 expression versus 30
months for those with high Smad7 expression (P Conclusion. Smad7 appears to be upregulated in
dermatial cancers compared to normal endometrium. Furthermore, high Smad7 gene
expression was associated with a shorter time to recurrence. Given that many endometrial
cancers have been shown to be TGF-[beta]-unresponsive, Smad7 should be investigated as a
potential target to restore TGF-[beta] responsiveness and limit tumor growth.

progression of human ovarian tumors in SCID mice." Gynecologic Oncology In Press, Corrected
Proof http://www.sciencedirect.com/science/article/B6WG6-4DFBSV4-
7/2/77a1e2e577385cc23f4a56f75b27bd57

Objective. Human ovarian carcinoma samples were orthotopically implanted into SCID mice to
investigate the contribution of matrix metalloproteases (MMPs) to the spread of ovarian
tumors. Methods. Mice were inoculated with patient tumor samples, and developed ovarian tumors
over a 16-week period with metastasis occurring in some mice. Species-specific quantitative RT-
PCR was used to identify the source of tumor-associated MMPs. Results. Membrane-type (MT)1-
MMP mRNA was significantly increased in high-grade tumors, tumors with evidence of serosal
involvement, and tumors in which distant metastases were detected. The increase in MT1-MMP
expression was predominantly from the human tumor cells, with a minor contribution from the
mouse ovarian stroma. Neither human nor mouse MT2-MMP were correlated with tumor
progression and MT3-MMP levels were negligible. While tumor cells did not produce significant
amounts of MMP-2 or MMP-9, the presence of tumor was associated with increased levels of
MMP-2 expression by mouse ovarian stroma. Stromal-derived MT1-MMP was greater in large
tumors and was associated with stromal MMP-2 expression but neither was significantly linked
with metastasis. Conclusions These studies indicate that tumor-derived MT1-MMP, more so than other gelatinolytic MMPs, is strongly linked to aggressive tumor behavior. This orthotopic model of human ovarian carcinoma is appropriate for studying ovarian tumor progression, and will be valuable in the further investigation of the metastatic process.


http://www.sciencedirect.com/science/article/B6WG6-4DFBSV4-7/2/77a1e2e577385cc23f4a56f75b27bd57

Objective Human ovarian carcinoma samples were orthotopically implanted into SCID mice to investigate the contribution of matrix metalloproteases (MMPs) to the spread of ovarian tumors. Methods Mice were inoculated with patient tumor samples, and developed ovarian tumors over a 16-week period with metastasis occurring in some mice. Species-specific quantitative RT-PCR was used to identify the source of tumor-associated MMPs. Results Membrane-type (MT1)-MMP mRNA was significantly increased in high-grade tumors, tumors with evidence of serosal involvement, and tumors in which distant metastases were detected. The increase in MT1-MMP expression was predominantly from the human tumor cells, with a minor contribution from the mouse ovarian stroma. Neither human nor mouse MT2-MMP were correlated with tumor progression and MT3-MMP levels were negligible. While tumor cells did not produce significant amounts of MMP-2 or MMP-9, the presence of tumor was associated with increased levels of MMP-2 expression by mouse ovarian stroma. Stromal-derived MT1-MMP was greater in large tumors and was associated with stromal MMP-2 expression but neither was significantly linked with metastasis. Conclusions These studies indicate that tumor-derived MT1-MMP, more so than other gelatinolytic MMPs, is strongly linked to aggressive tumor behavior. This orthotopic model of human ovarian carcinoma is appropriate for studying ovarian tumor progression, and will be valuable in the further investigation of the metastatic process.


http://www.sciencedirect.com/science/article/B6WG6-4BG8T8V-7/2/079d7315a426a1c5b9d7f69575389661

Objective. To determine whether squamous cervical cancers exhibit mutations or deletions in MHC class I genes or transport-associated protein (TAP) genes. Methods. Polymerase chain reaction based protocols were used to examine HLA class I and TAP genes in a panel of cervical tumours, using DNA from corresponding blood samples as controls. SSP-PCR protocols were similarly used for examination of all TAP alleles in tumour and blood samples. Results. In a series of cervical carcinomas, 7 of 27 (26%) exhibited mutations in HLA-A genes, while 12 of 23 (52%) exhibited mutations in TAP genes. HLA gene mutations were detected in 2 of 14 CIN2-3 lesions, and TAP gene mutations in none of 14, a frequency significantly less than observed in the cervical carcinoma samples (P P Conclusion. These data suggest that TAP genes may be relevant to evolution of cervical cancer from precursor lesions.

Objective. Data regarding signal transduction pathways in human tumors are largely confined to cell line studies to date. We have recently reported on the activation and prognostic role of mitogen-activated protein kinases (MAPK) in ovarian carcinoma in effusions. The objective of the present study was to investigate the expression and clinical role of dual-specificity phosphatases (DUSP), inhibitors of MAPK signaling, in ovarian cancer cells at this site. Methods. Thirty-nine fresh frozen malignant effusions from patients diagnosed with serous ovarian carcinoma were studied for mRNA expression of the DUSP MKP-1, MKP-4, MKP-5, and PAC-1 using RT-PCR. DUSP expression was analyzed for possible correlation with patient age, disease stage, tumor grade, histological grade, chemotherapy status, and survival. Results. MKP-1 and PAC-1 mRNA were found in 36 and 37 effusions, respectively, with expression levels showing considerable variation. MKP-4 and MKP-5 were uniformly absent. MKP-1 showed no association with clinicopathologic parameters. However, PAC-1 expression was significantly higher in effusions obtained before the institution of treatment with both platinum compounds (P = 0.029) and paclitaxel (P = 0.036). In univariate survival analysis, high level of expression of PAC-1 mRNA predicted significantly worse overall survival compared to low expression (mean = 30 vs. 52 months, MEDIAN = 25 vs. 46 months) (P = 0.007). Conclusions. Despite the limited size of this cohort, our results present the first evidence supporting a clinical role for PAC-1 in ovarian carcinoma. In view of the improved outcome associated with activation of all three MAPK families, as well as their elevated expression and activation in post-chemotherapy specimens presented in our previous work, they also suggest that PAC-1 is a true negative regulator of MAPK in ovarian carcinoma cells in effusions.


Objective. The likelihood of developing cervical cancer has been shown to be increased in persons with certain HLA alleles. We evaluated immune response genes in the HLA region of chromosome 6 to see if individual or interactive associations with cervical cancer risk could be identified. Methods. Tissue was obtained from 127 women undergoing surgical treatment for cervical cancer. Blood samples were obtained from 175 control subjects. A combination of polymerase chain reaction (PCR), sequence-specific PCR, and DNA sequencing was used to evaluate polymorphic alleles, including HLA class I B7, TNF [alpha], HLA class II DR2, TAP1, and TAP2 genes. Fisher’s exact test and logistic regression modeling were used for statistical analysis. Results. A significantly greater proportion of the patients with cervical cancer were found to have the HLA class II DR2 1501 allele (P = 0.023) and the TAP2 A/B heterozygous pattern of alleles (P = 0.0006) than were women without cervical cancer. A proportion of patients with cervical cancer significantly smaller than that of the control women had a polymorphism at the -238 position of the TNF promoter and the TAP1 C/C homozygous pattern of alleles. With logistic modeling, the markers that showed consistent association with the occurrence of cervical cancer were TAP2 A/B, HLA-DR2 1501, and TAP1 C/C. Conclusions. We demonstrated a significant association between immune response genes and the risk of cervical cancer. Our data create a compelling argument for a gene or a cluster of genes in the HLA region of chromosome 6 that regulates host immune responses to human papillomavirus infection in a manner that results in inherited susceptibility or resistance to the transforming properties of oncogenic papillomaviruses.

Vulvar intraepithelial neoplasia (VIN) is becoming more widespread and the patients are becoming still younger. Although progression to invasive vulvar carcinoma is uncommon, local recurrences are frequent and about one-quarter of the patients have multicentric genital disease. The aim of the present study was to search for a possible significant association of human papillomavirus (HPV) infection with vulvar carcinoma, recurrences, and multicentric disease. We used the polymerase chain reaction to examine vulvar and cervical biopsies from 43 patients with vulvar neoplasia for HPV type 16, which is the subtype most often detected in genital malignant or premalignant lesions. HPV 16 DNA sequences were found in 14 of 24 (58%) vulvar squamous carcinomas and in 15 of 19 (79%) VIN lesions. Nine patients (21%) had associated cervical neoplasia and six of these harbored HPV 16 in both lesions. Patients with recurrent intraepithelial neoplasia had a significantly higher incidence of HPV 16-positive lesions. No association was found with regard to the occurrence of multicentric disease or risk of malignant progression.


Fifty cervical adenocarcinomas and 50 squamous cell carcinomas from age-matched patients were examined for human papillomavirus (HPV) types 16 and 18. The polymerase chain reaction was used to examine formalin-fixed, paraffin-embedded carcinoma tissues for 120 and 113 bp sequences, respectively, of the highly conserved E6/E7 regions of the viral genomes. HPV type 16 was detected more often in squamous cell carcinomas than in adenocarcinomas (60% vs 18%, P < 0.001). These differences may reflect the fact that different virus receptors exist in cervical cells with different morphologic potential, or they may indicate that the specific HPV infection actually plays a role in directing carcinogenesis.


Objective. Topotecan, a novel topoisomerase-I inhibitor, is an active agent of second-line chemotherapy for extending the platinum-free interval (PFI) and improving the chances of a response to platinum in recurrent ovarian cancer patients. The aim of this study was to understand the molecular mechanism of topotecan-based second-line chemotherapy through an in vitro cell culture model and to gain clinical insight into sequencing issues for second-line treatment with novel agents versus retreatment with platinum. Study design. The human ovarian cancer cell line A2780 and the cisplatin resistance cell line A2780-CR were separately seeded in 6-well culture plates and then exposed to multiple concentrations of cisplatin plus paclitaxel or topotecan for 7 days. Surviving cells were recovered and cultured in drug-free media for 3 weeks and then replated in a 96-well microtiter plate. The LD50 for these cells was determined by a cytotoxic MTT assay after exposure to multiple clinically relevant concentrations of cisplatin or topotecan. Surviving cells were cultured in drug-free media for an additional 4 weeks at which
time the LD50 was reassessed for each cell population by a second MTT assay. Using RT-PCR and Northern blot hybridization to measure mRNA expression, the molecular profile of these cells in terms of resistance was evaluated for the multidrug-resistant gene (MDR-1), multidrug-resistant protein (MRP), Topoisomerase-I, and [beta]-Actin. Results. The LD50 to cisplatin was unchanged in A2780-CR cells treated by topotecan. Those A2780-CR cells originally exposed to higher concentrations of cisplatin became more resistant to cisplatin in the MTT assays, while those A2780-CR cell lines treated with a combination of lower cisplatin concentrations and paclitaxel became more sensitive to cisplatin in the MTT assay (P 50 for cisplatin in every cell line decreased significantly after a 4-week drug-free interval (P P P Conclusions. The acquired resistance to cisplatin in A2780 is potentially due to P-glycoprotein-mediated multidrug resistance. This acquired resistance to cisplatin is an unstable phenotype in that some cell populations become sensitive after a drug-free interval and topotecan treatment. This reversal of resistance, however, does not appear to be simply due to loss of MDR-1 expression. While in vivo confirmation is required, agents with novel mechanisms of action offer a strategy to extend the platinum-free interval and thereby improve survival in patients with recurrent ovarian cancer.


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Objective.(1) To identify and (2) validate genes that are up-regulated in ovarian cancer, and (3) to investigate whether the activity of a candidate gene, creatine kinase B (CKB) is elevated in pre-operative sera from ovarian cancer patients compared to patients with benign pelvic masses and normal controls. Methods. MICROMAX cDNA microarray system and RNA derived from pooled ovarian cancer cell lines and normal ovary surface epithelial cells (HOSE) were used to identify differentially expressed genes. Using RNA from both cell lines and from tissue obtained through laser capture microdissection (LCM), we performed quantitative PCR in order to validate up-regulation of one of these genes, creatine kinase B (CKB). Using a commercially available enzyme assay, CKB activity was measured in pre-operative serum samples obtained from 45 ovarian cancer patients, 49 patients with a benign pelvic mass, as well as 37 normal controls. Statistical analysis was preformed using an unpaired Student's t test. Results. Microarray technology revealed that CKB gene expression had a cancer to HOSE ratio of 18. RNA levels of CKB, measured by real-time PCR, were elevated a mean (and standard error) of 36-fold (8.4) in cancer cell lines compared with HOSE cells and 22.75-fold (10.45) in microdissected ovarian cancer epithelial cells compared with normal ovarian epithelial cells. In serum, the mean ([plus-or-minus sign]standard error) of CKB enzyme activity in cancer cases was 24.7 U/L units (15.8 (5.1)) compared to 9.6 U/L (5.6) for benign mass cases (P = 0.0088) and to 8.5 U/L (1.7) for normal controls (P = 0.0096). Conclusions. Microarray technology offers a method to identify tumor biomarkers with potential clinical usefulness. Our data indicated that CKB gene expression is up-regulated in ovarian cancer cells in vitro and in vivo and that CKB enzyme activity is significantly elevated in sera from ovarian cancer patients, including those with stage I disease. These findings suggest a potential role for CKB as a marker for early diagnosis.


http://www.sciencedirect.com/science/article/B6WG6-4D09FKH-1/2/fa109c8877bcfb2baa3296c3fb538840
Objective. Methylation of p16 promoter was evaluated in ovarian cancer to determine the role of p16 methylation in ovarian cancer prognosis.

Methods. Two hundred and forty-nine patients with primary epithelial ovarian cancer were selected for the study; these patients were followed for a median of 31 months. Genomic DNA extracted from fresh frozen tumor tissues were treated with sodium bisulfite and were analyzed for p16 methylation using methylation-specific PCR (MSP). Cox regression survival analysis was performed to examine the associations of p16 methylation with progression-free and overall survivals.

Results. Of the 249 patients, 100 (40%) were tested positive for p16 promoter methylation. The status of p16 methylation did not change significantly with patient age, disease stage, histological grade, residual tumor size, and debulking results, although p16 methylation seemed to occur more often in patients with advanced diseases or aggressive tumors. Compared to those without p16 methylation, patients with p16 methylation had significantly higher risk for disease progression ($P = 0.01$). The relative risk for progression was 1.69 (95% CI: 1.12-2.54), and the association remained statistically significant (RR = 1.54, 95% CI: 1.01-2.34) after adjusting for clinical and pathological variables. The risk for death was also higher in methylation positive patients than in methylation negative patients (RR = 1.33, 95% CI: 0.88-2.00), but the difference was not statistically significant.

Conclusion. The study suggests that promoter methylation in the p16 gene is associated with ovarian cancer progression, and evaluation of p16 methylation may have values in predicting ovarian cancer prognosis.


http://www.sciencedirect.com/science/article/B6WG6-4B2CB8B-9/2/9d0650ab96a4ab45800d29e33650634f

Objectives. The aim of this study was to detect and identify human papillomavirus (HPV) genotypes on a population of women infected by the human immunodeficiency virus (HIV) and to investigate the role of multiple infections on cervical dysplasia. Methods. Two hundred and fifty-five HIV-infected women were enrolled on a study to evaluate the prevalence of HPV and cervical intra-epithelial neoplasia (CIN). A group of HIV-negative women with confirmed CIN diagnosis was included for comparison. A polymerase chain reaction (PCR)-reverse hybridization method was applied to detect and precisely identify HPV types, specifically multiple infections. Results. On HIV patients, an altered Pap smear confirmed by biopsy was observed on 45 (18%); HPV-DNA prevalence was 87% (223/255), with 45% (116/255) infected by more than two types. In contrast, HPV-DNA was detected in all 36 women of the control group but only 3 were infected by more than two types. Cervical dysplasia was associated with low CD4 counts and elevated high-risk HPV viral load. However, the presence of multiple HPV types did not correlate with the degree of immune suppression or the presence of cervical lesions. Conclusions. Infection with multiple HPV types is a rather frequent finding on Brazilian HIV-infected women. On this population, concomitant infection with three or more HPV types does not seem to confer an additional risk of cervical dysplasia in comparison to single/double infections, nor to be related to more severe immunesuppresion.


http://www.sciencedirect.com/science/article/B6WG6-4DTTCJ1-1/2/c95f71cad86409bd60fcae94ea07b66a

Objectives. Functional assays of tumor suppression and loss of heterozygosity point to a tumor suppressor gene (TSG) for cervical cancer (CC) on chromosome 11q23. We evaluated IGSF4, a putative TSG located in the region, for promoter methylation and gene silencing in CC cell lines.
and cervical tissues. Methods IGSF4 expression was detected by both RT-PCR and Northern blot analysis. Methylation maps of the IGSF4 promoter region were generated for 11 CC cell lines based upon bisulfite-genomic sequencing, using seven nested-PCR primer sets covering 97 CpG sites. Methylation fingerprints in primary cervical tissues were evaluated by denaturing high performance liquid chromatography. Results A 4.4-kb mRNA was seen in cell lines, consistent with the RT-PCR results for both cell lines and primary cervical tissue. IGSF4 was expressed in 6/11 cell lines, 6/8 CC tissues and in all seven normal cervical epithelia. In the cell lines, IGSF4 silencing was associated with promoter hypermethylation. The methylation status in the region covering the -18 to -2 CpG sites correlated most strongly with expression, pointing to the existence of an unmethylated core in the IGSF4 promoter in cell lines expressing IGSF4. This unmethylated core spans approximately 180 bp and is immediately upstream of the ATG site. In primary tissues, methylation was detected in 15/23 (65%) CC specimens but in none of seven normal cervical epithelia. Conclusions Our data strongly suggest that IGSF4 is a TSG and that gene silencing by aberrant hypermethylation may contribute to the development of CC.


http://www.sciencedirect.com/science/article/B6WG6-48R1T3V-5/2/e07e9f5812a9bd612079a3e8d916651

Objective Despite the high prevalence of uterine leiomyoma in women, little is known about the pathophysiology of this tumor. This study intends to define the epigenetic modulation of this tumor. Methods Twenty-three pairs of leiomyomas and their adjacent myometria were collected. Status of DNA global methylation was determined by using DNA methyl acceptance assay and immunohistochemistry staining with 5-methylcytidine antibody. MRNA level of DNA methyltransferases (DNMT1, 3A, and 3B) was assessed by quantitative real time PCR. Results DNA global hypomethylation was detected in the leiomyoma tissues as compared with the adjacent myometria. DNMT1 expression was increased in 47.5% and was equal in 47.5% in leiomyomas compared to the adjacent myometria. On the other hand, over 74% of cases showed decreased expression of DNMT3A and 3B in leiomyomas compared to the adjacent myometria. Conclusion Global hypomethylation and imbalanced expression of DNMTs in uterine leiomyoma suggested a potential mechanism of epigenetic modulation in the development of this tumor.


http://www.sciencedirect.com/science/article/B6WG6-4DR1NPV-1/2/4670b8c4ca9022d47f08cf84c8370c68

Background To develop a simple and cost-effective method for the detection and genotyping of high-risk human papillomaviruses (HPV) using seminested polymerase chain reaction (PCR) and reverse hybridization. Methods Cervical swabs for HPV testing were collected from 127 women with normal cervical cytology and 57 patients with cervical lesions of various degrees. After DNA isolation, PCR amplification was first carried out using MY11 and MY09/HMB01 primers, then labeled by seminested PCR using the first PCR products and MY11/bioGP6† primers. One fifth of the second PCR products were resolved by gel electrophoresis. Genotyping for high-risk HPV was done separately, using the remaining products, by a high-risk HPV chip, which contained 13 type-specific oligonucleotides on a nylon membrane. The final result was detected by colorimetric
High-risk HPV DNA was detected in 19 (15%) of 127 women with normal cervical smear cytology, in 26 (89.7%) of 29 patients with cervical intraepithelial neoplasia (CIN), and in 27 (96.4%) of 28 patients with invasive cervical carcinoma. Multiple high-risk HPV infections were detected in five cases. HPV type 16 was the most frequent type of infection, comprising 34.5% and 53.6% of the patients with CIN and invasive carcinoma, respectively. The samples without a visible 190-bp band on electrophoresis exclusively showed negative hybridization results. This method could detect one to two copies of the HPV-16 genome derived from one SiHa cell. The overall sensitivity of HPV detection was 25 to 50 copies of HPV genome for each specimen. Thirteen high-risk types and twenty-four different types of HPV DNA showed specific hybridization without any cross-reaction.

Conclusions
Our results demonstrated the feasibility and optimistic prospects for this simple and cheap method of high-risk HPV genotyping. This technology can be easily set up in a routine molecular laboratory and would probably be of great value in cervical cancer prevention programs.


http://www.sciencedirect.com/science/article/B6WG6-4DVBGSG-4/2/029606d1d4b0f86d09526cd0692042d4a

Objective
In this study, genetic polymorphisms, NQO1 C609T, GSTM1 positive/null, and GSTT1 positive/null, were examined with reference to cervical cancer risk in a population-based incident case-control study in Japanese.

Methods
The cases comprised 131 cervical cancer patients: 87 cases with squamous cell carcinoma (SCC) and 44 with adenocarcinoma (ADC) or adenosquamous carcinoma (ADSC). Controls were sampled from 320 healthy women who underwent a health checkup.

Results
The cervical cancer risk was substantially elevated with smoking for all cases, SCC cases, and ADC/ADSC cases (OR = 4.50, 95% CI = 2.48-8.17, P = 0.032; respectively). The frequency of the NQO1 609TT genotype, reported to be associated with null enzyme activity, was higher in individuals with all cases and SCC than in the healthy controls (OR = 1.97, 95% CI = 1.06-3.66, P = 0.032; and OR = 2.42, 95% CI = 1.21-4.82, P = 0.012; respectively), but not in ADC/ADSC cases. Analysis of polymorphisms for GSTM1 and GSTT1 showed no significant differences between cervical cancer patients and controls. In stratification analysis, significant elevated risk of all cases and SCC was associated with the NQO1 609TT genotype among nonsmokers (OR = 2.15, 95% CI = 1.08-4.30, P = 0.030; and OR = 2.83, 95% CI = 1.21-6.31, P = 0.011; respectively), but not smokers. No gene-gene interaction was observed in our case subjects.

Conclusion
This is the first report that the NQO1 gene might be important in relation to the risk of squamous cell carcinoma of the cervix.


http://www.sciencedirect.com/science/article/B6WG6-49BY0WVF-2/06bb021cd0eb9f9af0e59537de2efa

Objective
Several tumors express the protein product of the protooncogene c-KIT. Some of these respond to imatinib mesylate, a tyrosine kinase inhibitor. The tumors that respond frequently have mutation(s) in exon 11 of c-KIT that encodes for the regulatory juxtamembrane helix. Some tumors that express KIT protein have mutation(s) in exon 17 of c-KIT; however, these do not respond to imatinib mesylate. This investigation was performed to determine the expression of KIT protein and mutational status of exons 11 and 17 of c-KIT in uterine sarcomas.

Methods
Twenty-five uterine sarcomas treated from 1990 to 2002 were evaluated.
These included 14 malignant mullerian mixed tumors (MMMT), 7 leiomyosarcomas (LMS), 2 endometrial stromal sarcomas (ESS), and 2 high-grade heterologous sarcomas (HGHS). Formalin-fixed, paraffin-embedded tissue sections were immunostained with anti-KIT antibody (Santa Cruz Biotechnology, Santa Cruz, CA) with a semiquantitative assessment. Normal myometrium when present in the section was used as an internal negative control. Areas of tumor were microdissected followed by DNA extraction, polymerase chain reaction (PCR) amplification of exons 11 and 17, single-strand conformational polymorphism (SSCP), and DNA sequencing to detect the presence of mutation(s).

Results All 25 tumors expressed KIT protein at varying levels as assessed by immunohistochemistry. The staining was diffuse and of moderate to strong intensity in 22 tumors. In three tumors (one of each type except MMMT) the staining intensity was weak. In MMMT the epithelial and sarcomatous foci stained similarly. No mutation(s) in exons 11 or 17 of c-KIT were identified in 24/25 tumors. One LMS had deletion of both exons 11 and 17.

Conclusion Although uterine sarcomas express KIT protein, they lack KIT-activating mutation(s) in exon 11 or 17 of c-KIT. Therefore, these tumors are unlikely to respond to imatinib mesylate.


http://www.sciencedirect.com/science/article/B6WG6-4DF46J8-H/2/23fa37f4dad317ee63e37e239bd9608d

Objectives Methylenetetrahydrofolate reductase (MTHFR) gene is associated with DNA hypomethylation, an established hallmark of human cancer cells. The aim of this study was to examine the effect of MTHFR polymorphism C677T on cervical carcinogenesis in the context of other environmental factors, such as smoking, parity, and age at the first intercourse.

Methods The study subjects were patients who were pathologically diagnosed with cervical neoplasia and who had a positive result for human papillomavirus (N = 462), and they were compared to normal healthy women as normal controls (N = 454). Genotypes of the patients and control samples were assayed by single base primer extension assay using SNapShot assay kit.

Results Compared with MTHFR C/C, the odds ratio (95% confidence interval) for MTHFR T/T was 1.4 (0.9-2.3) for invasive cervical cancer and 1.3 (0.8-2.3) for cervical intraepithelial neoplasia (CIN) II or III. The risks for invasive cervical cancer were higher with less than 40 years old at diagnosis (2.1, 1.0-4.3), than with over 40 years old at diagnosis (1.2, 0.7-2.2). Current smoking women with early onset with MTHFR T/T had a 4.7 (0.6-36.2) times higher risk of cervical cancer. The risks of MTHFR T/T or C/T also increased for women with an early age of first intercourse or for women with two or more children, as compared with MTHFR C/C.

Conclusion Polymorphisms of MTHFR are associated with a higher risk of developing cervical cancer, and in particular for an early onset of cervical carcinogenesis.


http://www.sciencedirect.com/science/article/B6WG6-4CN9MN-2/2/33e1a3748f17602a76e8beb29dcea4f4e

Objective. Human [alpha]-catenin gene (CTNNA1) on chromosome 5q31 is aberrantly expressed in various types of cancer including epithelial ovarian tumors. Allelic imbalance on this region has also been described in several malignant diseases. In the present work, the role of CTNNA1 as a candidate tumor suppressor gene was studied by comparing protein expression with allelic imbalance in human epithelial ovarian tumors.

Methods. [alpha]-Catenin protein expression was determined from two areas of 41 tumors, and tissues from these areas were microdissected. After
DNA extraction, AI analysis was carried out with eight microsatellite markers. Results. Altogether, 93% of the tumors (38 of 41) showed allelic imbalance at one or more loci. Two distinct common regions of allelic imbalance were identified, one comprising markers D5S2002 and D5S1995 and the other markers DSS393 and DSS476. Loss of the CTNNA1 gene did not appear to be involved in down-regulation of [alpha]-catenin in ovarian tumors, since allelic imbalance with a variety of markers, including CTNNA1 associated marker DSS476, was found in tumor samples independently of [alpha]-catenin expression. Furthermore, allelic imbalance analyses of two different samples from the same tumor revealed genetic heterogeneity. Conclusions. High allelic imbalance frequency indicates that chromosomal region 5q31 is functionally important in epithelial ovarian cancer. Allelic imbalance occurs at two distinct regions of which one includes the CTNNA1 gene. However, this gene is likely to be inactivated by mechanisms other than allelic imbalance. In addition, genetic heterogeneity observed in these tumors demonstrates the multiclonal nature of epithelial ovarian tumors.


http://www.sciencedirect.com/science/article/B6WG6-4BG8T8V-B/2/5f018e2f2ac3bd039aad6de2f8f3e80a

Objective. Leptin and its receptor are the key players in the regulation of energy balance and body weight control. However, their roles in gynecological malignancies are mostly unclear. In this study, we analyzed the expression and possible involvement of leptin and the leptin receptor in the pathogenesis of endometrial cancer. Methods. Radioimmunoassay was performed to analyze the serum leptin levels in the endometrial cancer patients, while RT-PCR, immunoblotting, and immunohistochemistry techniques were applied to study the expression of leptin receptor in the endometrioid-type endometrial cancer tissues. Furthermore, BrdU labeling followed by immunofluorescent analysis was used to analyze the effect of leptin receptor overexpression on endometrial cancer cell proliferation. Results. Serum leptin levels are elevated in endometrial cancer patients, but show no significant difference to those of normal controls when normalized by body mass index. On the other hand, lower expression levels of leptin receptor short form (Ob-Ra) were observed in most endometrial cancer tissues, especially in the poorly differentiated ones, and the forced expression of Ob-Ra in RL95-2 endometrial cancer cells prevented them from entering the S-phase. Conclusion. In summary, our data demonstrates for the first time that the leptin receptor is aberrantly expressed in endometrial cancer tissues and is possibly involved in the pathogenesis of endometrial cancer.

Hearing Research  (15)


http://www.sciencedirect.com/science/article/B6T73-49H73CH-1/2/03986cb3a52659a07b899f6054fa84d3

Hair cells are specialized mechanoreceptors common to auditory and vestibular sensory organs of mammalian and non-mammalian species. Different hair cells are believed to share common
features related to their mechanosensory function. It has been shown that hair cells possess various forms of motile properties that enhance their receptor function. Membrane-based electromotility is a form of hair cell motility observed in isolated outer hair cells (OHCs) of the cochlea. A novel membrane protein, prestin, recently cloned from gerbil and rat tissues, is presumably responsible for electromotility. We cloned prestin from mouse organ of Corti and confirmed strong homology of this protein among different rodent species. We explored whether or not prestin is present in hair cells of the vestibular system. Using reverse transcription-polymerase chain reaction, we demonstrated that prestin is expressed in mouse and rat auditory and vestibular organs, but not in chicken auditory periphery. In situ hybridization and immunolocalization studies confirmed the presence of prestin in OHCs as well as in vestibular hair cells (VHCs) of rodent saccule, utricle and crista ampullaris. However, in the VHCs, staining of varying intensity with anti-prestin antibodies was observed in the cytoplasm, but not in the lateral plasma membrane or in the stereociliary membrane. Whole-cell patch-clamp recordings showed that VHCs do not possess the voltage-dependent capacitance associated with membrane-based electromotility. We conclude that although prestin is expressed in VHCs, it is unlikely that it supports the form of somatic motility observed in OHCs.


http://www.sciencedirect.com/science/article/B6T73-478RSJ4-52/dbd40922ee3a3a3b8cbf342f118382d9

The type 1 vanilloid receptor (VR1) is a non-specific cation channel activated by capsaicin, lipoxygenase (LOX) products, heat and acid. This study demonstrates VR1 and 5-LOX expression by inner ear ganglion cells. A PCR product (210 bp) was amplified from both oligo(dT)- and random primer-generated cDNAs of rat spiral ganglion cells using VR1 gene-specific primers constructed from the 3' non-homologous region. This PCR product shared 100% sequence homology to a rat VR1 cDNA (GenBank accession no. AF029310) and a rat vanilloid receptor splice variant mRNA (GenBank accession no. AF158248). Frozen sections of PLP-fixed, decalcified Long-Evans rat temporal bones were stained immunohistochemically for VR1. Neurons and satellite cells in both the vestibular and spiral ganglia were VR1-immunopositive. Neurons and supporting cells in adjacent sections of these ganglia were immunopositive for 5-LOX. These findings raise the hypothesis that activation of VR1 by endogenous ligands may contribute to hypersensitivity of the eighth nerve to hair cell inputs in a variety of pathologic conditions, such as tinnitus, Meniere's disease and migraine. In particular, these data suggest that LOX activation during inflammatory processes or during cyclo-oxygenase inhibition (e.g. by aspirin) is a potential intrinsic source of VR1 activation in inner ear ganglia.


http://www.sciencedirect.com/science/article/B6T73-44MFJR7-32/4dc6b6b315651dc3b0ef9344339bd2bb

The recently manifested important role of the Ca2+-activated K+ channels, especially of the Slo gene-coded channels, for the cochlea function of the chicken raised the question of homolog expression in mammalian inner ear tissue. Molecular biological methods were used to demonstrate the expression of Ca2+-activated K+ channel subunits and splice variants of the Slo gene in the rat organ of Corti. RT-PCR experiments for the detection of rat Slo [alpha] subunit mRNA revealed the presence of several already known splice variants including variants which appeared to be typical for the organ of Corti (+58 aa) and for the brain (+61 aa). To detect the
accessory [beta] subunit we used Southern blot hybridization. Our data support the hypothesis
that Ca2+-activated K+ channel subunits (i.e. Slo variants) are also involved in the hearing of
mammals in the organ of Corti.

Fitzakerley, J. L., K. V. Star, et al. (2000). "Expression of Shal potassium channel subunits in the adult
http://www.sciencedirect.com/science/article/B6T73-411X9NB-3/2/4a5d722c1aeb33daa0300a9f111050be

The pattern of expression of potassium (K+) channel subunits is thought to contribute to the
establishment of the unique discharge characteristics exhibited by cochlear nucleus (CN)
neurons. This study describes the developmental distribution of mRNA for the three Shal channel
subunits Kv4.1, Kv4.2 and Kv4.3 within the mouse CN, as assessed with in situ hybridization and
RT-PCR techniques. Kv4.1 was not present in CN at any age. Kv4.2 mRNA was detectable as
early as postnatal day 2 (P2) in all CN subdivisions, and continued to be constitutively expressed
throughout development. Kv4.2 was abundantly expressed in a variety of CN cell types, including
all of the major projection neuron classes (i.e., octopus, bushy, stellate, fusiform, and giant cells).
In contrast, Kv4.3 was expressed at lower levels and by fewer cell types. Kv4.3-labeled cells were
more prevalent in ventral subdivisions than in the dorsal CN. Kv4.3 expression was significantly
delayed developmentally in comparison to Kv4.2, as it was detectable only after P14. Although
the techniques employed in this study detect mRNA and not protein, it can be inferred from the
differential distribution of Kv4 transcripts that CN neurons selectively regulate the expression of
Shal K+ channels among individual neurons throughout development.

by polymerase chain reaction (PCR) and in situ hybridization." Hearing Research 78(2): 175.
http://www.sciencedirect.com/science/article/B6T73-485PD2X-6N/2/dd8dd1d9c127230d5dcce06e2370795

Expression of mineralocorticoid type I receptor (MR) gene in the rat cochlea was determined
using molecular biological techniques. We synthesized complementary DNA (cDNA) from rat
cochlear total RNA and then amplified MR cDNA fragments by polymerase chain reaction (PCR).
The amplified cDNA fragments were subcloned into an expression vector and the nucleotide
sequence was analyzed to confirm the expression of mRNA encoding MR in the cochlea. We
then synthesized digoxigenin-labeled riboprobes with this cloned DNA template and examined
the localization of MR mRNA in the cochlea by in situ hybridization. The amino acid sequence of
MR cDNA expressed in the cochlea was identical to that of the MR first cloned in the rat
hippocampus. In situ hybridization showed the expression of MR mRNA in marginal cells of the
stria vascularis, suggesting that aldosterone may regulate microhomeostasis of the endolymph,
presumably by modulating Na, K-ATPase activity. Intense MR signal was also identified in spiral
ganglion cells, the function of which remains to be determined.

Gong, T.-W. L., A. D. Hegeman, et al. (1996). "Identification of genes expressed after noise exposure in
http://www.sciencedirect.com/science/article/B6T73-3W0NHBW-3/2/4fa3c41313d5b743058b124471348469
We used differential display of mRNA, a method based on reverse transcriptase-PCR, to identify genes whose expression increases in response to acoustic trauma in the chick basilar papilla. Identifying these genes would provide insight into processes involved in repair of the damaged epithelium or in hair cell regeneration. We compared mRNA from the basilar papilla of normal chicks, from chicks exposed to an octave band noise (center frequency: 1.5 kHz) presented at 118 dB for 6 h, and from chicks exposed to noise and allowed to recover for 2 days. Thus far, we have identified 70 bands that appear to be differentially displayed on DNA sequencing gels; approximately 40 of these bands have been subcloned and sequenced. DNA sequences were compared with sequences in the GenBank database to identify genes with significant (70-85%) sequence identity to known genes. Chick cDNAs identified included: the parathyroid hormone-related protein, an immediate early gene; the [delta]-subunit of the neuronal-specific Ca2+/calmodulin-regulated protein kinase II; and the GTP-binding protein CDC42, a member of the ras superfamily of G proteins. A fourth cDNA had 84% sequence identity to an uncharacterized human cDNA (expressed sequence tag), indicating that this is a novel gene. Slot-blot hybridization analysis of these cDNAs probed with labeled DNA generated from mRNA from each experimental group indicated higher levels of mRNA for each of these four genes after noise exposure. These results indicate the potential involvement of both Ca2+/calmodulin-mediated signaling and GTPase cascades in the response to noise damage and during hair cell regeneration in the chick basilar papilla.


http://www.sciencedirect.com/science/article/B6T73-48KFP8-1/2/fcdb750fedb5515acdb6381dc2a442b

A recessive deafness mutation in the mouse arose spontaneously and was identified in a colony segregating a null allele of the gastrin-releasing peptide receptor (Grpr) locus. Auditory-evoked brain stem response measurements revealed deafness in 7-week-old affected mice. By linkage analyses, the mutant phenotype was mapped near marker D10Mit186 and the protocadherin gene PcDH15. As shown by complementation testing, the new mutation is allelic with Ames waltzer (PcDH15av). Sequencing mutant-derived brain PcDH15 cDNAs identified the insertion of a cytosine residue at nucleotide position c2099 (2099insC), which results in a frame-shift and premature stop codon. Abnormal stereocilia on inner and outer hair cells of the organ of Corti were identified by scanning electron microscopy as early as postnatal day 0 and cross-sectional histology revealed severe neuroepithelial degeneration in cochleas of 30-50-day-old mutants. The new allele of Ames waltzer, designated PcDH15av-Jfb, may aid in studying the histopathology associated with Usher syndrome type 1F, which is caused by a functional null allele of PCDH15.


http://www.sciencedirect.com/science/article/B6T73-46TF5J-1/2/efedf09ba986af0c5b897c64ca51

Biotinidase deficiency is an autosomal recessively inherited disorder characterized by neurological and cutaneous features, including sensorineural hearing loss. Although many of the features of the disorder are reversible following treatment with biotin, the hearing loss appears to be irreversible. To better characterize the nature of the hearing loss in this disorder, location of the expression and presence of biotinidase within the brain was examined using Northern blot analysis, in vitro hybridization of a cDNA panel, and immunohistochemical staining. Results indicate low, but detectable expression of biotinidase throughout the brain, but increased
concentrations of biotinidase within the dorsal cochlear nucleus, ventral cochlear nucleus, and superior olivary complex of the brainstem, as well as, in the hair cells and spiral ganglion of the cochlea. These findings suggest that biotinidase and possibly biotin plays an important role in hearing.


http://www.sciencedirect.com/science/article/B6T73-4DTKMHW-3/2/19c6795177cd553828a7746ab4bd0567

The transient receptor potential cation channel subfamily V (TRPV) is a non-specific cation ion channel receptor family that is gated by heat, protons, low extracellular osmolarity and arachidonic acid derivatives. Since some of these endogenous agonists of TRPV receptors are reactive oxygen intermediates produced by lipoxygenases, it has been hypothesized that some members of the TRPV family may respond to challenges by reactive oxygen species. This study used real-time PCR to quantitatively track changes in TRPV1-4 mRNA expression in the spiral, vestibular, and trigeminal ganglia and the kidney from kanamycin (KM)-treated mice. TRPV1, TRPV2, TRPV3 and TRPV4 mRNAs were expressed in spiral and vestibular ganglia, and TRPV2 and TRPV1 mRNAs were most predominant in control mice. After KM (700 mg/kg s.c. b.i.d., 14 days), TRPV1 mRNA and protein expression were significantly up-regulated both in the spiral and vestibular ganglia, but expression was unaffected in the trigeminal ganglion and kidney. Real-time PCR also demonstrated a significant down-regulation in TRPV4 mRNA expression in the inner ear ganglia and kidney after KM treatment. All these mRNA and protein expression changes were eliminated by simultaneous administration of dihydroxybenzoate (300 mg/kg s.c. b.i.d., 14 days), an anti-oxidant that blocks KM ototoxicity. It is proposed that up-regulated TRPV1 expression during KM exposure may promote ganglion cell survival by contributing to neuronal depolarization, with KM-induced tinnitus and dizziness as consequences.


http://www.sciencedirect.com/science/article/B6T73-4CTD19J-1/2/87fe07b77fbb058cb31504a2ab0129bb

Uncoupling proteins (UCPs) are a proton transporter family located in the mitochondrial inner membrane. The molecular expression and activity of UCPs in brown adipose tissue and skeletal muscle are regulated by factors as diverse as chronic overeating and cold exposure, suggesting roles in energy expenditure and heat production. Although UCP2, UCP4 and brain mitochondrial carrier protein-1 (BMCP-1, i.e. UCP5) mRNAs are expressed in the central nervous system, their central function is unknown. This study presents the first evidence on localization and quantitative expression of UCPs in the rat inner ear by real-time PCR and immunohistochemistry. Real-time PCR studies revealed that UCP2 mRNA was expressed in the vestibular and spiral ganglia more abundantly than any other UCP. Neocortex, by contrast, contained UCP2 and UCP4 equally. Notably, UCP3 and UCP4 mRNAs were expressed in inner ear ganglia, but brain UCP3 mRNA expression level was undetectable by simple PCR. Immunohistochemical studies confirmed that both UCP2- and UCP3-like immunoreactivities were detected in vestibular and spiral ganglion cells and co-localized with a mitochondrial marker, MitoFluorGreen. According to previous reports, UCP2 and UCP3 are thermogenic in yeast and brain UCP2 has been suggested to modulate pre- and post-synaptic events by axonal thermogenesis. It has also been reported recently that UCP2 and UCP3 responses to superoxide application may be an antioxidant.
A protective mechanism. Therefore, it is suggested that mitochondrial UCPs (UCP2, UCP3, UCP4) may play both a protective role against oxidative damage and a thermal signaling role in the eighth nerve.


http://www.sciencedirect.com/science/article/B6T73-3T3TMDP-C/2/3e716af9a6f56cd7fb0fcb2eeec7e8538

Although mechanisms regulating inner ear fluid have not been yet elucidated, control of blood flow has been thought to be of great importance. Vasoactive intestinal polypeptide (VIP) was the first neuropeptide demonstrated in cerebrovascular nerves. To study the possible role of VIP in regulation of inner ear fluid, we investigated the presence of mRNA for VIP and VIP receptor in the rat inner ear using a reverse transcription-polymerase chain reaction (RT-PCR) method. A single band of the size expected for VIP and its receptor was detected in mRNA from the rat inner ear by using primers specific for VIP and the receptor. The nucleotide sequences of the subcloned RT-PCR products were identical to those of rat VIP and the rat lung VIP receptor. These results indicate that both VIP and VIP receptor are expressed in the inner ear of the rat and suggest that VIP may be implicated in regulation of fluid in the inner ear.


http://www.sciencedirect.com/science/article/B6T73-4F1503H-2/2/7b2637f754b2d0c14120716303b05de

Mutations in the Connexin 26 (Cx26) gene (GJB2) are a common cause of hereditary hearing impairment. We report the identification of a novel point mutation in the Cx26 gene, Leu205Pro(L205P), linked to familial, autosomal recessive sensorineural hearing loss. This missense mutation, causing amino acid leucine at position 205 to be substituted by proline, is located in the highly conserved sequence of the fourth transmembrane domain (TM4) of Cx26. Hearing loss with this mutation occurred in a Georgian Jewish family, was congenital, moderate to profound and nonprogressive. We have shown that the new mutation L205P in Cx26 is strongly associated with congenital NSHL. Multiple-sample screening for this mutation can be easily performed with a mismatch PCR that creates a restriction site.


http://www.sciencedirect.com/science/article/B6T73-485V0XS-2/2/933a07d5c5b0f346914e86b27f7ddf9

Levels of expression of mRNAs encoding the different Ephs and ephrins were measured by semi-quantitative reverse-transcription polymerase chain reaction in developing mouse whole inner ears, and in dissected fractions of the neonatal mouse inner ear. Nineteen of the 24 known Ephs and ephrins were surveyed. The results showed that between embryonic age (E) 11.5 days and E12.5, levels increased 10-300 times per unit of tissue. In neonatal mice, the fraction containing combined organ of Corti and spiral ganglion showed relatively strong expression of EphA4, EphB3, ephrin-A3, ephrin-B2 and ephrin-B3. In the lateral wall, EphA4, ephrin-A3 and ephrin-B2
were strongly expressed, while ephrin-A3 was particularly strongly expressed in utricular and sacular sensory epithelia. The results suggest that the Ephs and ephrins are likely to play a part in the differentiation of the structures of the inner ear, and show which Ephs and ephrins are most likely to play important roles in the different structures.


http://www.sciencedirect.com/science/article/B6T73-42WX3VB-6/2/e1dc4770c512ad277bf3b92a8cadab97

Four different fibroblast growth factor receptors (FGFR) are known, three of which have splice variants (known as the b and c variants) in the FGF-binding domain, to give different patterns of sensitivity to the different FGFs. The expression of the b and c variants of the FGF receptors, together with the expression of the ligands FGF1, FGF2, FGF3, FGF7, FGF8b and FGF8c, was determined by quantitative reverse transcription-polymerase chain reaction in developing whole mouse inner ears, and in dissected components of the postnatal mouse inner ear. At embryonic age (E)10.5 days, when the otocyst is a simple closed sac, the receptor most heavily expressed was FGFR2b, relative to the postnatal day 0 level. Over the period E10.5-E12.5, during which the structures of the inner ear start to form, the expression of the different FGF receptors increased 102-104 fold per unit of tissue, and there was a gradual switch towards expression of the 'c' splice variants of FGFR2 and FGFR3 rather than the 'b' variants. At E10.5, the ligands most heavily expressed, relative to the postnatal day 0 level, were FGF3, FGF8b and FGF8c. In the postnatal inner ear, the patterns of expression of receptors and ligands tended to be correlated, such that receptor variants were expressed in the same regions as the ligands that are known to activate them effectively. The neural/sensory region expressed high levels of FGFR3c, and high levels of the ligand FGF8b. The same area also expressed high levels of FGFR1b and FGFR2b, and high levels of FGF3. The lateral wall of the cochlea (including the stria vascularis and the spiral ligament) expressed high levels of FGFR1c and FGF2. It is suggested that the different FGF receptors and ligands are expressed in a spatially coordinated pattern, to selectively program cochlear development.


http://www.sciencedirect.com/science/article/B6T73-42WX3VB-G/2/42550fd61174a8af615f222720019925

Glial cell line-derived neurotrophic factor (GDNF) is a survival factor for many neuronal cell types which signals through a heterodimer receptor consisting of GDNF-family receptor [alpha] 1 (GFR[alpha]-1) and Ret (arranged during ransformation). GDNF expression has previously been reported in the inner hair cells of the rat cochlea, with expression of GFR[alpha]-1 but not Ret in the cell bodies of the auditory nerve (spiral ganglion cells), using in situ hybridization. The present study used reverse transcription-polymerase chain reaction (RT-PCR), and immunocytochemistry to examine GDNF, GFR[alpha]-1 and Ret in the adult rat auditory nerve. Semi-quantitative RT-PCR showed expression of GDNF and the two receptor components, GFR[alpha]-1 and Ret, in the modiolar subfraction of the cochlea containing spiral ganglion cells. A shorter mRNA splice variant for GDNF was also detected. Immunocytochemistry showed immunostaining in the modiolus for GDNF, GFR[alpha]-1 and Ret that was confined to spiral ganglion cells. When RT-PCR expression levels were compared to the expression in the substantia nigra, GFR[alpha]-1 expression levels were similar, Ret mRNA was lower in the modiolus and GDNF expression was
higher in the modiolus. However, when GDNF was further assessed using Western blot, while GDNF protein was found in the modiolus it was at lower levels than in substantia nigra tissue. These results demonstrate that GDNF and both of its receptor components are found in spiral ganglion cells of the adult rat cochlea. Along with the previous report of GDNF in inner hair cells, these new results provide a basis for the role of GDNF as a survival factor for the auditory nerve, as suggested by previous studies.

**Heart** (2)


http://heart.bmjournals.com


http://heart.bmjournals.com/cgi/content/abstract/90/1/13

Intrauterine and neonatal manifestations of congenital long QT syndrome are associated with a high cardiac risk, particularly when atrioventricular block and excessive QT prolongation (> 600 ms1/2) are present. In a female newborn with these features, treatment with propranolol and mexiletine led to complete reduction of arrhythmia that was maintained 1.5 years later. High throughput genetic analysis found a sodium channel gene (LQT3) mutation. Disappearance of the 2:1 atrioventricular block and QTc shortening (from 740 ms1/2 to 480 ms1/2), however, was achieved when mexiletine was added to propranolol. This effect was considered to be possibly genotype related. Early onset forms of long QT syndrome may benefit from advanced genotyping.

**Hepatology** (3)


http://www.sciencedirect.com/science/article/B6WG8-4CMYXTW-19/2/41d1a04a8adfb0f4b99d246bb11eae

The p53 gene is frequently mutated in human tumors; in hepatocellular carcinomas, there is a high frequency of a specific mutation at codon 249 in regions with significant aflatoxin exposure. To assess the role of this p53 mutation in the development of hepatocellular carcinoma, a mutant murine p53 gene, p53ser246, which corresponds to human codon 249, was transfected into a
differentiated, nontransformed hepatocyte cell line AML12. Expression of p53ser246 in this line resulted in a growth advantage when compared with either a control vector (which contains a large p53 deletion) or with a different p53 mutant, val135, not found in hepatocellular carcinoma. Overall, there was a threefold increase in colony formation after transfection with p53ser246 as compared with the control or p53val135 vectors, and the p53ser246 plates developed consistently larger colonies. Whereas clones expressing the control or p53val135 constructs showed no significant morphological changes, clones expressing p53ser246 showed increased heterogeneity (large multinucleated cells and areas of small crowded cells) without focus formation. In addition, the ser246 mutation imparted a growth advantage in serum free media, suggesting less dependence on specific factors present in serum. None of the mutant p53 or control lines were capable of growth in soft agar or tumor formation in nude mice. Thus in this model, in which endogenous wild-type p53 expression is retained, a high level of mutant p53 expression is not sufficient to transform hepatocytes. Our findings indicate that p53ser246 has effects on hepatocytes that may result in a clonal growth advantage and suggest that additional factors are required for the development of hepatocellular carcinoma.


http://www.sciencedirect.com/science/article/B6WG8-4CNT1SW-8C/2/605aeae652d53db81844704c838d64a33

One hypothesis is that postnatal liver growth involves replication of mature hepatocytes, which have an unlimited proliferative potential. An alternative viewpoint is that only certain periportal cells can replicate extensively and that daughter cells stream slowly from the periportal to the pericentral region of the liver. Transgenic mice expressing the beta-galactosidase ([beta]-gal) gene from the human al antitrypsin promoter were used to examine the proliferative potential of hepatocytes. Surprisingly, only 10% of hepatocytes in two different transgenic lines stain blue with X-gal. In neonatal animals, singlets or doublets of expressing cells are randomly scattered throughout the liver. Although the overall frequency of blue cells is similar in older animals, these cells are present in much larger clusters, suggesting that individual expressing cells have replicated to form a clonally derived cluster. Expression patterns are not altered by the administration of an acute phase stimulus or by the performance a partial hepatectomy, suggesting that the expression state cannot be easily altered, and making it more likely that the expression state is indeed fixed. These results suggest that the clusters of blue cells are clonally derived in the transgenic mice. They argue that the parenchymal hepatocyte is responsible for growth in the postnatal liver and that streaming of liver cells does not occur.


http://www.sciencedirect.com/science/article/B6WG8-4CNT2X9-27/2/3031a328a32718be6096b1d4c98aebf9

Reovirus type 3 has been implicated in the origin and pathogenesis of extrahepatic biliary atresia and idiopathic neonatal hepatitis, but routine detection of this virus in hepatobiliary tissues from affected infants by culture and histological techniques has been unsuccessful. In this study, oligonucleotide primers specific to the M3 genome segment of reovirus 3 (Dearing) were used in a reverse transcriptase--mediated polymerase chain reaction technique to develop a sensitive and specific assay for the detection of reovirus 3 RNA in formalinfixed, paraffin-embedded patient samples. Optimal reaction conditions were determined by testing infected murine tissues and
preserved human liver tissue supplemented with reovirus 3. Archival specimens from 50 infants, including 14 with extrahepatic biliary atresia, 20 with idiopathic neonatal hepatitis, and 16 age-matched controls, were evaluated. Successful amplification of human albumin complementary DNA from the preserved tissues confirmed the presence of intact RNA in every patient specimen tested. Analysis of the amplification reactions by agarose gel electrophoresis and Southern blot hybridization detected the presence of reoviral RNA only once in a single patient sample. These results do not support a strong role for reovirus 3 in the development of neonatal cholestatic liver disease. The recent association of other RNA viruses of the Reoviridae family with murine liver disease and human extrahepatic biliary atresia indicates that continued investigation into a viral cause for idiopathic neonatal hepatobiliary disease is warranted.

**Hepatology Research (19)**


http://www.sciencedirect.com/science/article/B6T74-3VH81JC-8/2/03e5e68daa24cb4f87526157b2c89b33

Although hepatitis C virus (HCV) detection by polymerase chain reaction (PCR) assay is now the standard, extensive clinical application has been thwarted by the troublesome procedure, long reaction time and potential for contamination. To overcome these problems, we carried out a PCR assay for the detection of HCV, hepatitis G virus (HGV) and hepatitis B virus (HBV) genomes directly from serum samples without any nucleic acid extraction (direct PCR). The sensitivity of this assay was one chimpanzee-infectious dose of HCV and a 10-1 chimpanzee-infectious dose of HBV. This result was similar to the sensitivity determined by the conventional PCR. Furthermore, when the detection rate of these genomes in serum samples from chimpanzees and humans is compared, the results matched completely between two different PCR assays. The whole process, including the reverse transcriptase reaction and second round PCR, can be completed within 6 h by the combination of the direct PCR and one-step PCR assay. Our findings indicate that this method is simple, rapid and highly sensitive and could be useful for the screening of blood-borne hepatitis virus infections using serum samples.


http://www.sciencedirect.com/science/article/B6T74-46SFD5F-2/2/07a1b45f9570a99189ea0108a3bb6598

During the follow-up of 19 patients with self-limited acute hepatitis B for more than 2 years, clearance of hepatitis B surface antigen from the sera was observed in all patients within 6 months after disease onset, and the corresponding antibody (anti-HBs) appeared in 17 of the 19 patients within 12 months. However, upon performing nested polymerase chain reaction with the estimated sensitivity of 120-200 copies/ml, using two independent pairs of primers derived from the well-conserved sequences in the S gene or C gene region of the hepatitis B virus (HBV)
genomes of all seven genotypes, HBV DNA was detected over a period of at least 12 months in serum samples obtained from five (26%) of the 19 patients, although it became undetectable in all five patients at 2-3 years after disease onset. The titer of antibody against hepatitis B core antigen (anti-HBc), assayed by the hemagglutination inhibition (HI, 2N) test, was significantly lower at the initial examination in the five patients who remained viremic for at least 12 months, than in the remaining 14 patients who cleared HBV DNA from their sera within 12 months after disease onset (10.6+/2.7 vs. 13.6+/0.7, PP<0.01). These results indicate that the initial titer and dynamics of anti-HBc may reflect the evolution of HBV viremia after clinical recovery from acute hepatitis B.


http://www.sciencedirect.com/science/article/B6T74-3YRW0DF-4/2/3a335a4ddb4a543ae82bddaed80fcf70

To elucidate the role of hepatitis viruses in the pathogenesis of Behcet's disease (BD), we measured hepatitis viral markers (anti-hepatitis A (anti-HA), HBsAg, anti-HBs, anti-HBc) and viral nucleic acids (hepatitis B virus (HBV)-DNA, hepatitis C virus (HCV)-RNA, GB virus C (GBV-C)-RNA, TT virus (TTV)-DNA) in the sera of 68 BD patients along with 76 blood donors matched for age and sex. Positivity of anti-HA in patients with BD (36.8%) was lower than that in blood donors (68.0%). Both anti-HCV and HCV-RNA were detected in only one (1.5%) patient with BD and in none of the blood donors. The prevalence ratios of HBsAg, anti-HBs, anti-HBc in both groups were similar (2.9:0, 16.2:15.8 and 17.7:19.7%, respectively). However, serum HBV-DNA was detected more frequently in BD patients (8/68; 11.8%) than in blood donors (2/76; 2.6%) (P<0.05). The prevalence of GBV-C-RNA was also higher in patients with BD (4/68; 5.9%) compared with blood donors (0%). However, characteristics and clinical features are similar between GBV-C-RNA-positive and -negative groups. With respect to the prevalence of TTV-DNA, there was no significant difference between BD patients (23.5%) and blood donors (30.3%). Our study indicates that HBV and GBV-C infection might be related to BD, although the role of these viruses remains to be investigated.


http://www.sciencedirect.com/science/article/B6T74-485PJ0B-1/2/e3f761f50d067adef285bda0f2042c2c

The aim of this work was to study the induction and secretion of interleukin 8 (IL-8) and some oxidative stress parameters after ethanol (EtOH), acetaldehyde (Ac) or lipopolysaccharide (LPS) treatment on HepG2 cells. Cells were treated with 50 mM EtOH, 175 [mu]M Ac or 1 [mu]g/ml of LPS. IL-8 induction and secretion were determined in the presence of the toxics, and the effect of antioxidants N-acetyl--cysteine and 1,1,3,3-tetramethyl-2-thiourea was evaluated. Further, the effect of adding polyclonal anti-human tumor necrosis factor [alpha] (TNF-[alpha]) and H2O2 was studied, and catalase, superoxide dismutase and glutathione peroxidase activities were determined. Lipid peroxidation increased significantly only in Ac-treated cells. All toxics failed to decrease significantly the intracellular levels of reduced GSH. Catalase activity was diminished in all treatments, while other enzyme activities did not present changes. No change in peroxide production was found with any treatment. IL-8 secretion increased in Ac (41%) and in LPS (38%)-treated cells. Antioxidant and anti-TNF-[alpha] treatments decreased IL-8 secretion. H2O2 (0.25 mM)-treated cells increased IL-8 secretion. IL-8 reverse transcriptase-polymerase chain reaction results correlated with secretion values. Our results show that Ac and LPS treatment produced an
increased IL-8 induction and secretion. Oxidative stress and TNF-[alpha] are mediators in IL-8 response. This observation suggests that in the in vivo liver, the mechanism of ethanol-induced IL-8 production requires ethanol metabolism, and hepatocytes do not require the interaction among different populations of liver cells to respond.


http://www.sciencedirect.com/science/article/B6T74-47GYK8P-5/2/3e4fc9f100a5e052217993600dac8ec5

Recently, we identified TTV isolates from nonhuman primates and named them simian TTV (s-TTV). To investigate the prevalence of s-TTV in humans, we examined sera from healthy individuals and patients with liver diseases in Japan for the presence of s-TTV DNA by PCR assay. s-TTV DNA was determined by nested PCR using s-TTV-specific primers designed from untranslated region of s-TTV genome. s-TTV DNA sequence was detected in three of 200 (1.5%) healthy adults but none of 48 infants without liver disease. On the other hand, s-TTV DNA was detected in 30 of 287 (10.5%) Japanese patients with liver disease. s-TTV coinfection with hepatitis B virus and hepatitis C virus were present in 16.7 and 30% of these patients, respectively, while 53.3% of patients were positive for s-TTV alone. Nucleotide sequence analyses in 20 patients confirmed that these PCR products were derived from s-TTV genome sequences and nearly 85% identical to those of s-TTV prototypes from chimpanzees. Phylogenetic analysis demonstrated that all s-TTV isolates from humans were distinguished clearly from the human TTV isolates. Furthermore, s-TTV in humans was classified into two different genotypes as well as simians. Our results indicate that generally 10.5% of Japanese patients with liver diseases were infected with s-TTV. The routes of s-TTV transmission from animal to human require clarification.


http://www.sciencedirect.com/science/article/B6T74-3TXKWX7-5/2/1df8d1e1b41f27a4b71af674d596d17f

A transfusion-transmissible agent, designated hepatitis G virus (HGV), was recently identified. We have cloned the full-length nucleotide sequence of the HGV genome (denoted HGV-IM71) recovered from a Japanese patient with liver cirrhosis. The HGV-IM71 isolate was composed of 9387 nucleotides (nt) with 5' and 3' untranslated regions of 458 and 310 nt, respectively. It had a single open reading frame, encoding 2873 amino acid (aa) residues. This isolate differed from previously reported HGV/GBV-C isolates by 8-15% of the nucleotide sequence and 3-5% of the amino acid sequence. The isolate lacked a clearly identifiable core gene that had only 47 aa residues. Based on phylogenetic analysis of the full-genome sequence, it was concluded that HGV-IM71 belonged to the Asia type (type 3) of HGV genotypes.


http://www.sciencedirect.com/science/article/B6T74-4DT2M3R-1/2/5d2588b3f6a028d2b5056d326c952bb7
Background: Hepatitis B virus (HBV) has been classified into seven genotypes (A-G). HBV genotypes have a geographically characteristic distribution. Since HBV genotype G (HBV/G) was identified recently, little is known about the distribution of HBV/G in Japan. The aim of this study was to clarify this issue.

Patients and methods: Seven hundred and twenty-one serum samples obtained from patients with HBV in Japan were investigated. The patients included 149 asymptomatic carriers, 325 with chronic hepatitis, 129 with liver cirrhosis, and 118 with hepatocellular carcinoma. Six HBV genotypes (A-F) were determined by restriction fragment length polymorphism targeting to the S region of the HBV genome. Furthermore, HBV/G was investigated by polymerase chain reaction with hemi-nested primers derived from an HBV/G-specific nucleotide sequence.

Results: Of the 721 serum samples investigated, 12 subjects were classified as having HBV/A, 88 HBV/B, 610 HBV/C, 3 HBV/D, and 1 HBV/F. Seven subjects had a mixed infection with distinct genotypes, two with HBV/A and HBV/D, and five with HBV/B and HBV/C. HBV/G was not identified among the 721 samples.

Conclusion: HBV/G was not identified in a large cohort of patients with HBV, either single or dual infection. HBV/G seems to be an extremely rare genotype in Japan.


http://www.sciencedirect.com/science/article/B6T74-46VBSFM-5/2/b1530e3c7928e92e293378dc0807b17f

Accumulating evidence suggests the presence of latent hepatitis B virus (HBV) infection in the liver of individuals negative for HBV surface antigen (HBsAg) but positive for antibodies to HBV core antigen (anti-HBc) at low titer. It remains unclear, however, whether positive anti-HBc in the serum invariably reflects latent HBV infection. In this study, we examined the presence of HBV genomes in the liver tissue of 33 donors and 30 recipients of living-related liver transplantation with positive for anti-HBc together with time course changes in their anti-HBc titer. None of these anti-HBc-positive healthy donors or recipients was positive for HBV-DNA nor anti-HBc at high titer (200 dilution) in their sera. However, HBV-DNA was detected in the liver tissue of 24 of 33 healthy anti-HBc-positive donors (72.7%) and five of 30 anti-HBc-positive recipients (16.7%). Interestingly, anti-HBc was continuously positive in all healthy donors tested. In contrast, anti-HBc titers in 75% of recipients, who were positive for anti-HBc at the time of liver transplantation, gradually decreased after the operation, and finally became negative after the mean follow up period of 9.0 months (range 1.2-45.1). Notably, HBV-DNA was never detected in the liver of those recipients who were transiently positive for anti-HBc. In conclusion, our findings suggested the possibility that presence of circulating anti-HBc does not always reflect the presence of HBV genomes in the liver tissue of anti-HBc-positive patients.


http://www.sciencedirect.com/science/article/B6T74-3SY2XKK-7/2/a5c244b0871cf81acb7de4f6957e9a4c

Inactivation of the p53 tumor suppressor gene is considered to occur as a late event involved in hepatocarcinogenesis. Its detection is thought to provide a useful information for the clinical management of hepatocellular carcinomas (HCCs). A rapid screening test was devised for allelic loss of the p53 gene in samples obtained by needle biopsy using non-radioisotopic (RI) microsatellite analysis combined with the microdissection method for clinical laboratory. Biopsy
materials from 23 HCCs were examined to detect loss of heterozygosity (LOH) of the p53 gene after use for a histopathological diagnosis. Two microsatellite loci in the p53 gene were amplified by the polymerase chain reaction (PCR) using DNA extracted from microdissected cells, and amplified DNA fragments were subjected to non-RI detection using silver staining. More than 40-50 microdissected cells from a formalin-fixed paraffin-embedded tissue are enough to amplify both alleles of the p53 gene during the PCR. The combination of the two polymorphic microsatellite markers encompassed 60% of Japanese patients as informative. This method has detected LOH of the p53 locus in 54% of informative primary HCCs. Furthermore, LOH of the p53 gene was frequently detected in more advanced HCC as to the histological grade and clinical stage as shown in the previous report. The system for rapid and safe detection of the p53 gene allelic loss should provide useful information on the strategy for the treatment of HCC in the clinical laboratory.


http://www.sciencedirect.com/science/article/B6T74-42MW5SX-7/2/2e6a6dc4367d99eaf0e5f56a8124190d

Information about M6P/IGF2R and p53 genes in hepatocarcinogenesis is limited and controversial. We tested the loss of heterozygosity (LOH) of M6P/IGF2R and p53 genes in cirrhotic and neoplastic foci in surgically resected livers of 30 patients with hepatocellular carcinoma (HCC). The DNAs extracted from microdissected specimens were used for polymerase-chain-reaction (PCR)-based assay. LOH of the M6P/IGF2R gene in the primary HCCs was detected in 10 of 22 informative cases (45%). In five of these 10 cases (50%), LOH was detected in cirrhotic lesions adjacent to the HCCs. The allelic loss patterns of M6P/IGF2R in liver cirrhosis (LC) were identical to those in the corresponding HCC, suggesting that HCC could develop from one of the cells in which M6P/IGF2R had been lost. Furthermore, LOH of the p53 gene in HCC was detected in 10 (43%) of 23 informative cases, and p53 loss in cirrhotic foci adjacent to HCC was shown in one of the 10 cases (10%). The pattern of allelic loss of the p53 gene in the cirrhotic foci was identical with that in the corresponding tumor. The LOH of the M6P/IGF2R and p53 genes occurred independently in HCCs. LOH of the M6P/IGF2R locus was a relatively frequent and possibly early event in hepatocarcinogenesis, and LOH of the M6P/IGF2R gene and LOH of the p53 gene occurred independently.


http://www.sciencedirect.com/science/article/B6T74-42HX7SX-2/2/29b2e23cba5160fc5a2b6f007ac2ab7

TT virus (TTV) has been reported to occur in association with elevated alanine aminotransferase (ALT) levels in patients with posttransfusion hepatitis of unknown etiology. We examined whether the presence, change of DNA titer, or variation in sequence of this virus is associated with acute or chronic liver dysfunction in Japanese. We detected TTV by polymerase chain reaction (PCR) using primers generated from the conserved region of the TTV genome. Direct DNA sequencing of the original N22 region was used to characterize TTV isolates. We detected TTV DNA in 15 (25%) of 60 patients with liver dysfunction. Variants recovered from infected patients formed four genotypes/subtypes, corresponding to G1a, G1b, G2, and G4. Although TTV DNA titers in patients with G2 and G4 were lower than those with G1, TTV was consistently detected regardless of genotype/subtype. TTV infection continued for at least 1 year after normalization of
ALT level in patients with acute liver dysfunction. Changes in DNA titer, substitutions of deduced amino acids, and variety of quasispecies of TTV were detected during the observation period, but no significant fluctuation in ALT level was found. We conclude that persistent infection, changes in DNA titer, and variation in sequence of this novel virus are not significantly related to hepatic disorders.

http://www.sciencedirect.com/science/article/B6T74-45XXF0F-7/2/7afe3c6755dcd9981b50803983a8cb8b

Acute ethanol administration temporarily decreases the sensitivity to endotoxin (lipopolysaccharide, LPS) in the liver. The purpose of this study was to investigate the changes of toll-like receptor (TLR)-4, a newly identified LPS receptor in macrophages, in the liver following acute ethanol administration. Male C57BL/6N mice were given a bolus intragastric administration of ethanol (5 mg/g BW) through a gastric canula, and liver samples were obtained 2-48 h later. RAW264.7 macrophages were cultured in the presence of ethanol (100 mM) or LPS (10 ng/ml) for up to 4 h. TLR-4 mRNA in the liver and RAW264.7 cells was detected by RNase protection assay. As expected, TLR-4 mRNA was clearly detected in the control liver; however, it was barely detectable in the liver 2-6 h after ethanol administration, followed by the gradual increase to the basal levels 48 h later. Interestingly, LPS (10 ng/ml), but not ethanol (100 mM), decreased TLR-4 mRNA in RAW264.7 macrophages in 4 h. Indeed, gut-sterilization by oral antibiotics pretreatment prevented the decrease in TLR-4 mRNA caused by acute ethanol administration, supporting the hypothesis that gut-derived endotoxin is involved in the mechanism. These findings clearly indicated that acute ethanol administration in vivo down-regulates TLR-4 expression in the liver. This phenomenon most likely explains the mechanism by which acute ethanol blunts the response of Kupffer cells to LPS transiently.

http://www.sciencedirect.com/science/article/B6T74-3W3N8M6-7/2/3a30996f8ff6c2227d14061f14904fda

Prevalence and disease association of the TT virus (TTV) were studied in Japanese patients with various types of viral hepatitis. A total of 317 patients with viral hepatitis were analyzed, and the results were compared to those of 100 apparently healthy controls. TTV DNA in serum was measured by semi-nested polymerase chain reaction. Prevalence of TTV DNA was significantly higher in patients with hepatitis A (36%, 5/14), hepatitis B (35%, 35/101), hepatitis C (61%, 90/148), and non-A-E hepatitis (41%, 22/54) than in healthy controls (12%, 12/100), respectively. In each type of hepatitis, the prevalence did not differ between acute and chronic liver diseases, and did not increase with the complication of hepatocellular carcinoma. The clinical backgrounds did not differ between TTV DNA positive and negative patients, in patients with acute hepatitis or in those with chronic liver diseases. Similarly, no liver function test showed a significantly higher level of in TTV DNA positive patients than in negative ones. In conclusion, TTV infection was highly prevalent in patients with viral hepatitis, especially in those with hepatitis C. TTV was suggested to have a weak pathogenicity (or no pathogenicity), at least when co-infecting with an established hepatitis virus.

http://www.sciencedirect.com/science/article/B6T74-4C4X4K6-1/2/7a436120b263a92b728652af50c7041c

The aim of this study was to estimate the correlation between telomerase activity and the expression of human telomerase RNA component (hTERC), human telomerase reverse transcriptase (hTERT) in patients with hepatocellular carcinoma (HCC), and to analyze the influence of the nucleotide homology of the hTERC template region on telomerase activity. Six HCC patients and two chronic hepatitis patients were enrolled in this study. Telomerase activity was determined using the fluorescence-based telomeric repeat amplification protocol (TRAP) method. Quantification of hTERC and hTERTmRNA was performed using a real-time PCR method. Furthermore, a portion of the hTERC gene was amplified using nested RT-PCR methods. After sub-cloning, the nucleotide sequence of the cloned hTERC that contained the template region was determined. Telomerase activity and hTERTmRNA was detected in all cancerous tissues, while hTERC was present in both tumorous and non-tumorous lesions. The level of telomerase activity correlated with expression of hTERTmRNA, but not that of hTERC. The nucleotide sequence of cloned hTERC was similar in both tumorous and non-tumorous lesions. The expression of hTERT may be a definitive factor in the activation of telomerase in hepatocarcinogenesis.


http://www.sciencedirect.com/science/article/B6T74-3YJYCPK-2/2/2a6f347739de553784a6d693ea1513bc

We employed a PCR assay system TaKaRa Ex Taq(TM) (heat-resistant DNA polymerase), which has 3'-5' exonuclease activity to increase the sensitivity for TT virus (TTV) DNA detection. Sera obtained from 95 hepatitis B virus carriers without hepatitis C virus coinfection were tested for TTV DNA and the sensitivity of this assay system was compared with the PCR systems reported previously. Of the 95 individuals, TTV DNA was identified in 14 (14.7%) with the PCR reported by Nishizawa et al., in 66 (69.5%) with the PCR reported by Okamoto et al., in 80 (84.2%) by our assay system, and in 86 (90.5%) with the PCR reported by Takahashi et al. Phylogenetic analysis of nucleotide sequences amplified by the PCR revealed that genotypes 1a, 1b, 2, 3, 4, 5 were amplified efficiently by our assay system, while only a part of TTV DNA clone of genotype 1a was amplified by the PCR reported by Nishizawa et al. The prevalence of circulating TTV DNA became higher with age. These results indicate that our assay system with TaKaRa Ex Taq(TM) has confirmed high prevalence of TTV infection and that at least five genotypes prevail in Japan. In addition, acute TTV infection is supposed to cause long-standing viremia.


http://www.sciencedirect.com/science/article/B6T74-45XXF0F-4/2/998d14ead57822112b3113abe86415db

The platelet count increases after a sustained response to interferon (IFN) treatment for chronic
hepatitis C (CH-C). However, the extent of the increase differs by patient. We investigated whether concurrent TT virus (TTV) infection interferes with the improvement of thrombocytopenia. Serial serum samples were obtained from 85 noncirrhotic CH-C patients who achieved a sustained virologic response for hepatitis C virus (HCV) upon IFN treatment, and tested for TTV DNA by three polymerase chain reaction (PCR) methods (UTR, N22 and TTV genotype-1). UTR PCR can detect essentially all TTV genotypes, whereas N22 PCR primarily detects four major TTV genotypes (1-4). Eighty-four patients (84/85, 99%) were positive for TTV DNA by UTR PCR, 27 (32%) by N22 PCR and 18 (21%) by TTV genotype-1 PCR just before IFN treatment was started (baseline). A sustained virologic response for TTV was observed in 6% (5/84) by UTR PCR, 52% (14/27) by N22 PCR and 56% (10/18) by TTV genotype-1 PCR. The platelet count was significantly lower in the N22 PCR-positive group than in the N22 PCR-negative group not only at baseline (14.9+/−3.8 vs. 18.1+/−6.4 x 10⁴/μl, P<0.05), the differences also being statistically significant by TTV genotype-1 PCR, but not by UTR PCR. These results suggest that certain TTV genotypes including genotype 1 may play a role in aggravating the thrombocytopenia of CH-C patients, either alone or in concert with HCV.


A molecular epidemiological survey of various hepatitis viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV), was carried out in Ho Chi Minh City, Vietnam. This study included of 295 patients with liver disease (234 viral related and 61 non-viral related) and 100 healthy individuals. The infection rates of HBV and HCV in 234 liver disease patients with acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, were 31.2 and 19.2%, respectively. On the other hand, detection rates of these viruses in healthy populations were 10 and 2%, respectively (P<0.005 and <0.0001, respectively). None of cases tested was positive for HDV RNA. The most common viral genotypes were type B and C of HBV (43 and 57%) and type 2a of HCV (33.3%). Surprisingly, high prevalence of HBV pre-S2 deletion mutant was found in 22 of 87 (25.3%) patients with chronic liver disease. Moreover, antibody to hepatitis E virus (HEV) immunoglobulin G (IgG) was detected in 78 of 185 (42%) and IgM in 1 of 185 (0.5%) patients. The age prevalence of anti-HEV IgG was reached 61.9% in 21-40-year-olds. These results suggest that these hepatitis viruses, except for HDV, are spreading among liver disease patients in Ho Chi Minh city, Vietnam and HBV was the most important causative agent correlated with liver disease in this area.

IgM was detectable after alanine transaminase levels were elevated and TTV DNA was detectable in the patients. The duration of the anti-TTV IgM was short-lived compared with anti-TTV IgG. Anti-TTV IgM was not detected in sera from any of 36 healthy individuals, with no detectable anti-TTV IgG or TTV DNA in their serum. These results indicate that anti-TTV IgM antibodies would be a useful marker to detect acute TTV infection.


http://www.sciencedirect.com/science/article/B6T74-48BC6B8-6/2/7a165c790931130366bc12435bfac8aa

The endoglycosidase heparanase plays an important role in tumor cell invasion, angiogenesis and metastasis. In this study, we analyzed heparanase expression in primary hepatocellular carcinoma (HCC), liver cirrhosis and normal liver tissues to further evaluate its role in tumor pathogenesis. Heparanase mRNA expression was measured by real-time quantitative RT-PCR and localized at the cellular level by in situ hybridization. Heparanase protein levels and its localization were determined by Western blotting and immunohistochemistry. Expression of heparanase mRNA in primary HCC was increased 2-fold compared with liver cirrhosis and 2.2-fold in comparison with normal liver tissues, and this overexpression was even more pronounced in advanced stage HCC. In contrast, heparanase expression levels between cirrhotic tissues and normal liver tissues were not significantly different. In HCC the increased heparanase expression was localized in hepatic tumor cells and was only weakly present or absent in normal hepatocytes, bile duct epithelial cells and the connective tissues. These results suggest that increased heparanase expression is involved in the pathogenesis and progression of HCC. Its specific up-regulation in HCC but not in liver cirrhosis indicates that it might be used as a molecular marker for the differentiation of these disorders.

Hum. Reprod. (16)


http://humrep.oupjournals.org/cgi/content/abstract/18/10/2039

BACKGROUND: We recently showed that vascular endothelial growth factor (VEGF) expression by endometrial glandular epithelial and stromal cells, and endometrial microvascular endothelial cell permeability, an early step in angiogenesis, were rapidly increased by estradiol (E2) administration to ovariectomized baboons. We proposed that estrogen promotes endometrial angiogenesis by regulating VEGF expression by glandular epithelial and stromal cells. In the present study, we developed a co-culture of human endometrial cells and microvascular endothelial cells to determine whether the regulatory role shown for estrogen on endometrial angiogenesis in vivo in the non-human primate would be demonstrable in vitro in the human. METHODS AND RESULTS: Human endometrial glandular epithelial and stromal cells were co-cultured with human myometrial microvascular endothelial cells (HMMECs) and E2. HMMEC tube formation (means ± SEM, % endothelial tube area/total endothelial cell area), an index of angiogenesis, was 65% (P < 0.05) and 2-fold (P < 0.01) greater in cells co-cultured with human
glandular epithelial cells (54 +/- 7%) and glandular epithelial cells plus E2 (66 +/- 11%), respectively, compared with medium (33 +/- 4%). In contrast, endothelial tube formation was not altered in HMMECs incubated with endometrial stromal cells (32 +/- 4%), stromal cells plus E2 (36 +/- 2%) or E2 (39 +/- 3%). CONCLUSIONS: We propose that estrogen, by regulating expression and secretion of angiogenic factors such as VEGF by glandular epithelial cells of the endometrium, regulates endometrial angiogenesis.


http://humrep.oupjournals.org/cgi/content/abstract/deh852v1

BACKGROUND: Vascular endothelial growth factor (VEGF), a major mediator of angiogenesis and vascular permeability, is known to play a key role in the pathophysiology of endometriosis.

METHODS AND RESULTS: The single nucleotide polymorphisms, -460C>T and +405G>C, in the 5'-untranslated region of the VEGF gene were tested for association in a case-control study of 215 affected women and 210 women with no evidence of disease. All the women were of South Indian origin and ascertained from the same infertility clinic. The genotype and allele frequencies of the -460C>T polymorphism did not differ significantly between cases and controls. In contrast, the genotype (P=0.002) and allele (P=0.001) frequencies of the +405G>C polymorphism showed a significant difference between cases and controls. The +405 GG genotype was found more often in patients with an endometrioma >3 cm compared to controls. The frequency of the -460T/+405C haplotype (P=0.016) was significantly lower in affected women compared to controls. CONCLUSIONS: The -460T/+405C haplotype in the VEGF gene, which is associated with lower promoter activity, was significantly less common in women with endometriosis than in controls. These data suggest that the +405G allele may influence the likelihood of a woman developing the disease.


http://humrep.oupjournals.org/cgi/content/abstract/20/5/1235

BACKGROUND: Aneuploidies involve [-~80% of chromosomal anomalies found in spontaneous miscarriages. Since cytogenetic studies show high rates of failure, we have incorporated the quantitative fluorescent polymerase chain reaction (QF-PCR) technique to the study of numerical chromosome anomalies in miscarriages. METHODS: Multiplex and simple QF-PCR assays have been performed on 160 miscarriage and 34 parental DNA samples analysing specific short tandem repeat (STR) markers for chromosomes 2, 7, 13, 15, 16, 18, 21, 22 and X. Cases successfully karyotyped were used as controls in our study. RESULTS: While maternal contamination could be detected in such cases, a molecular result was obtained for 94% of miscarriages without a cytogenetic one. Thirty-six per cent of them were diagnosed with numerical chromosome anomalies. Parental origin of the extra chromosome and the error stage of meiosis could be also determined. CONCLUSIONS: QF-PCR represents a useful and reliable tool to diagnose aneuploidies in spontaneous miscarriages. It provides information about parental and meiotic origin of anomaly, allowing an appropriate genetic counselling.

http://humrep.oupjournals.org/cgi/content/abstract/17/8/2073

BACKGROUND: The relationship between Chlamydia trachomatis tubal factor infertility (TFI) and the host's immunoregulatory genes was studied. METHODS: Cell-mediated immune responses to C. trachomatis and chlamydial heat shock protein (CHSP60) were determined by lymphocyte proliferation assay. HLA-DQ alleles and interleukin-10 (IL-10) promoter polymorphism (-1082 A/G) were analysed in 52 TFI cases and in 61 controls by PCR. RESULTS: HLA-DQB1 or DQA1 alleles did not significantly differ between the TFI group and the control group. However, DQA1*0102 and DQB1*0602 alleles together with IL-10 -1082AA genotype were found significantly more frequently in the TFI patients than in the controls (0.18 and 0.02 respectively; P = 0.005). Five (22%) of the 23 patients who had a positive lymphocyte proliferative response to CHSP60 were positive also for IL-10 -1082AA and for the HLA-DQA1*0102 and HLA-DQB1*0602 alleles. CONCLUSIONS: Our results reveal an association of a cellular immune response to CHSP60, HLA class II alleles and IL-10 promoter genotypes in patients with chlamydial TFI.


http://humrep.oupjournals.org/cgi/content/abstract/20/2/536

BACKGROUND: Age at menopause is under strong genetic control. So far, genetic variations of only one gene, the PvuII polymorphism of the estrogen receptor (alpha) (ER{alpha}) gene, have been shown to be associated with age at onset of menopause. This study aims to investigate whether PvuII, XbaI and B-variant polymorphisms of the ER{alpha} gene, and the MspAI polymorphism of the cytochrome P450c17(alpha) (CYP17) gene are associated with age at menopause in a Dutch cohort. METHODS: DNA was isolated from urine samples of 385 Caucasian women with natural menopause and the genotypes of the four polymorphisms were determined. A questionnaire was used for background characteristics. The genotypes of PvuII, XbaI, MspAI were obtained by PCR restriction fragment length polymorphism analysis. The B-variant was determined with an allele-specific oligonucleotide hybridization method. Two-sided t-tests were performed to assess the association between the four polymorphisms and menopausal age. The PvuII and XbaI polymorphisms were analysed separately as well as in a combined score. RESULTS: The results show that none of the polymorphisms independently, nor the combined genotypes for PvuII and XbaI, were associated with age at natural menopause. CONCLUSION: No evidence was found for a relationship between common variants of the ER{alpha} gene and the CYP17 gene with age at natural menopause.


http://humrep.oupjournals.org/cgi/content/abstract/19/9/2084

BACKGROUND: Polymorphism in the CTG triplet number in the myotonic dystrophy type 1 (DM1PK) gene has been proposed as being associated with idiopathic azoospermia. The aim of this study was to investigate whether the CTG trinucleotide amplification in the DM1PK gene is associated with male subfertility. METHODS: We evaluated 107 subfertile patients, male partners
of infertile couples, affected by non-obstructive azoospermia (n=38) and oligoasthenoteratozoospermia (OAT) (n=69), and 102 men with proven fertility. Main outcome measures were CTG repeat size in the DM1PK gene, testicular volume, sperm concentration, rapid progressive motility, normal morphology, serum FSH levels, testicular histology and Johnsen score. RESULTS: In subfertile males, no minimal mutation or mutation carriers were found. The difference in the number of CTG repeat lengths between the groups was not statistically significant (P=0.825). There was no correlation between the number of CTG repeats and the clinical parameters of subfertile patients: testicular volume, sperm concentration, rapid progressive motility, normal morphology, FSH level, testicular histology and Johnsen score. CONCLUSIONS: The number of CTG repeats in the normal or mutational range of DM1PK gene is associated with neither idiopathic male subfertility nor with clinical characteristics of male subfertility.


BACKGROUND: The adverse effects of hydrosalpinx on the outcomes of IVF have been well documented, but the mechanisms of hydrosalpinx fluid formation remain unclear. This study compares the mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (KDR and flt-1) in the hydrosalpinx with that in the healthy oviduct. METHODS: Oviduct tissue was collected from 10 infertile women with hydrosalpinx and 10 parous women with healthy oviduct. The mRNA expression of VEGF and its receptors in isolated oviduct epithelial cells were analysed using semi-quantitative reverse-transcriptase PCR. RESULTS: mRNA expression of VEGF and its receptor flt-1 in the hydrosalpinx was significantly higher than that in the healthy oviduct, but no significant difference was demonstrated for the KDR receptor. CONCLUSIONS: This study supports the notion that VEGF may play an important role in the hydrosalpinx fluid formation, possibly by promoting vascular and epithelial permeability and therefore serum transudation.


BACKGROUND: The zona pellucida (ZP) is an extracellular glycoprotein matrix which surrounds all mammalian oocytes. Recent data have shown the presence of four human zona genes (ZP1, ZP2, ZP3 and ZPB). The aim of the study was to determine if all four ZP proteins are expressed and present in the human. METHODS: cDNA derived from human oocytes were used to amplify by PCR the four ZP genes. In addition, isolated native human ZP were heat-solubilized, trypsin-digested and subjected to tandem mass spectrometry (MS/MS). RESULTS: All four genes were expressed and the respective proteins present in the human ZP. Moreover, a bioinformatics approach showed that the mouse ZPB gene, although present, is likely to encode a non-functional protein. CONCLUSIONS: Four ZP genes are expressed in human oocytes (ZP1, ZP2, ZP3 and ZPB) and preliminary data show that the four corresponding ZP proteins are present in the human ZP. Therefore, this is a fundamental difference with the mouse model.

BACKGROUND: The zona pellucida (ZP) has multiple roles in reproductive processes, including oocyte maturation, fertilization and implantation. We used, for the first time, a genetic approach to study whether human ZP genes possess structural alterations in women with unsuccessful IVF trials. In theory, this may result in gradual reduction of sperm-zona interaction and eventually in total fertilization failure (TFF). METHODS: Eighteen infertile women (TFFs) whose IVF did not result in any fertilized oocytes, whereas fertilization by ICSI was successful, were screened for mutations in ZP genes by means of conformation-sensitive gel electrophoresis. Twenty-three fertilizers in IVF (FIVFs) and 68 women with proven fertility (WPFs) constituted the two control groups. RESULTS: Altogether, 20 sequence variations were found in the ZP genes. Two variations in ZP3, one in the regulatory region (c. 1-87 T[&gt;G) and one in exon 6 [c. 894 G[&gt;A (p. K298)] existed more frequently in TFFs than in FIVF and WPF groups (P-values 0.027 and 0.008, respectively). CONCLUSIONS: Our study on ZP genes of infertile women revealed a high degree of sequence variations. This may reflect gradual reduction of fertility among TFFs, but the putative roles and influences of single variations can only be hypothesized.


BACKGROUND: We report the first attempts at preimplantation genetic diagnosis (PGD) and IVF and their accompanying difficulties for achondroplasia (ACH) patients. METHODS: A PGD test was developed using fluorescent single cell PCR on lymphoblasts from patients and controls and from blastomeres from surplus IVF embryos. A specific digestion control based on the use of two fluorochromes was elaborated. Ovarian stimulation and oocyte retrieval were carried out using conventional protocols. RESULTS: We performed 88 single cell tests for which amplification was obtained in 86 (97.7%) single lymphoblasts. Allele drop out (ADO) was observed in two out of 53 (3.7%) heterozygous lymphoblasts. If we combine the results from the blastomere testing from surplus embryos with those from PGD cycles and re-analysis after PGD, we obtained a PCR signal in 84% of cases of which 91% were correctly diagnosed at the G380 locus. A total of six cycles were performed resulting in three embryo transfers. We observed difficulties in ovarian stimulation and oocyte retrieval with affected female patients. No pregnancy was obtained. CONCLUSION: A PGD test for ACH is now available at our centre but our initial practice raises questions on the feasibility of such a test, specially with affected female patients.


BACKGROUND: Preimplantation genetic diagnosis (PGD) usually involves blastomere biopsy 3 days post-insemination (p.i.), followed by genetic analysis and transfer of unaffected embryos later on day 3 or 4. We evaluate a strategy involving embryo biopsy on day 3 p.i., genetic analysis on day 4 and, following culture in blastocyst sequential media, transfer of unaffected embryos on day 5 p.i. METHODS: PGD cycles were initiated in 15 couples at risk of transmitting (beta)-thalassaemia major. Oocyte retrieval and ICSI were performed according to standard protocols. Embryo culture used blastocyst sequential media. Embryos were biopsied on day 3 p.i. using acid
Tyrode’s for zona drilling, and the single blastomeres were genotyped by a protocol involving nested polymerase chain reaction and denaturing gradient gel electrophoresis analysis.

RESULTS: Forty of 109 (37%) embryos biopsied on day 3 p.i. developed to blastocysts by day 5 p.i., with at least one blastocyst available for transfer in 12 cycles (80%). Genotype analysis characterized 51/109 (47%) embryos unaffected for (beta)-thalassaemia major, of which 28 were blastocysts. Transfer of 37 day 5 p.i. embryos (blastocysts and non blastocysts) initiated eight clinical pregnancies. Implantation rate per embryo transferred was 12/37 (32%). CONCLUSIONS: Embryo biopsy on day 3, followed by delayed transfer until day 5 p.i. offers a novel and effective strategy to overcome the time limit encountered when performing PGD, without compromising embryo implantation.


http://humrep.oupjournals.org/cgi/content/abstract/19/11/2648

BACKGROUND: We investigated the relationship between idiopathic recurrent pregnancy loss (RPL) and genetic polymorphisms in phase I and phase II detoxification genes which include CYP1A1, CYP2D6, GSTM1, GSTP1 and GSTT1. METHOD: A case-control study comprised 160 females with RPL and 63 healthy controls with a successful reproductive history. RESULTS: The CYP1A1 variant allele was present at frequencies of 0.61 and 0.44 in cases and controls, respectively (odds ratio = 1.93; P=0.023, 95% confidence interval 1.10-3.38). The CYP2D6 variant allele was present at a frequency of 0.17 in females with RPL, while in the control population the frequency was 0.16. The GSTM1 and GSTT1 null genotypes were present at frequencies of 0.39 and 0.26 in RPL cases, whereas in controls the frequencies were 0.37 and 0.17, respectively. The mutant GSTP1 frequencies in case and control women were 0.38 and 0.40, respectively. We report a significant association of the CYP1A1*2A allele with RPL which is confirmed by logistic regression analysis. No association was observed for the other polymorphisms or in their combinations studied. CONCLUSIONS: The present study suggests the occurrence of the CYP1A1*2A allele as a probable risk factor in idiopathic recurrent miscarriages.


http://humrep.oupjournals.org/cgi/content/abstract/20/4/974

BACKGROUND: Endometriosis, an estrogen-dependent disease, is believed to be influenced by multiple genetic and environmental factors. Here, we evaluated whether the risk and severity of endometriosis are associated with polymorphisms in estradiol-synthesizing enzyme genes: the Ser312Gly polymorphism in 17-beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) and the Arg264Cys polymorphism in cytochrome P450, subfamily XIX (CYP19). METHODS: All participants underwent diagnostic laparoscopy, and the stage of endometriosis was determined according to the Revised American Fertility Society classification. Of the 138 women enrolled, 59 had no endometriosis, 21 had stage I, 10 had stage II, 23 had stage III and 25 had stage IV. SNPs were discriminated by allele-specific oligonucleotide hybridization. RESULTS: Individuals having at least one A-allele (A/G or A/A genotype) of HSD17B1 showed a significantly increased risk of endometriosis (A/G genotype: adjusted OR, 3.06; 95%CI 1.21-7.74; A/A genotype: adjusted OR, 3.02; 95%CI 1.08-8.43). There was a significant trend associating A/G + A/A genotypes with severity of endometriosis (P for trend <0.01). No statistically significant association was found for the CYP19 polymorphism. CONCLUSIONS: Evidence for association between the Ser312Gly polymorphism in HSD17B1 and endometriosis was found in a Japanese
population. The A-allele of HSD17B1 appears to confer higher risk for endometriosis.


http://humrep.oupjournals.org/cgi/content/abstract/18/2/267

BACKGROUND: Cytokines have been described to play a major role in the pathogenesis of idiopathic recurrent miscarriage (IRM). We investigated the association between IRM and a polymorphism of the interleukin-6 (IL-6) gene and IL-6 serum levels. METHODS: In a prospective case-control study, we studied 161 women with IRM and 124 healthy controls. Peripheral venous puncture, DNA extraction and PCR were employed to genotype women for the presence of a polymorphism at position -174 in the promoter region of IL-6. Serum IL-6 levels were assessed by a commercially available ELISA. RESULTS: Allele frequencies among women with IRM and controls were 63.4 and 58.1% respectively for allele G (wild type), and 36.6 and 41.9% respectively for allele C (mutant). No association between allele C and the occurrence of IRM was found (odds ratio 0.8; 95% confidence interval = 0.57-1.12; P = NS). IL-6 serum levels were not significantly different between genotypes and between the study and control groups. CONCLUSIONS: This is the first report on an IL-6 polymorphism in IRM. Although known to alter IL-6 expression, the IL-6 polymorphism investigated was not associated with IRM and alterations in IL-6 serum levels in a Middle-European Caucasian population.


http://humrep.oupjournals.org/cgi/content/abstract/19/3/700

BACKGROUND: (beta)-Thalassaemia results from co-inheritance of two mutant (beta)-globin alleles. Allogeneic cord blood cell transplantation (CBT) from an HLA-identical sibling donor is an excellent treatment option for (beta)-thalassaemia. In families with an affected child and willing to have another child, IVF followed by preimplantation genetic diagnosis (PGD) can be applied to exclude affected embryos. Furthermore, healthy embryos could be HLA matched with the affected child so that cord blood from the future newborn can be used to transplant the affected sibling. METHODS: We developed an indirect single-cell HLA typing technique based on the use of a bank of seven microsatellite markers within the HLA locus from which four informative and evenly distributed markers were selected. RESULTS: The methodology was validated in three (beta)-thalassaemia families having six ovarian stimulation cycles in view of IVF and PGD. Six PGD cycles were performed in two families. On 58 embryos tested, the combined PCR was successful in 54 (93%). Two transfers were done and one clinical pregnancy was obtained. Using confirmatory analysis on 50 embryos, the accuracy for HLA typing was 100%. CONCLUSION: This strategy offers a new therapeutic option for patients with (beta)-thalassaemia and other monogenic diseases that can be cured with CBT.


http://humrep.oupjournals.org/cgi/content/abstract/19/11/2653
BACKGROUND: In view of evidence suggesting an immunological cause of recurrent spontaneous abortions (RSA) and the large number of maternal natural killer (NK) cells present in the pregnant uterus, we investigated the genetic polymorphism of the killer cell immunoglobulin-like receptors (KIR) in women with RSA. METHODS: KIR gene repertoire and KIR2DL4 (a receptor for HLA-G) genotyping were determined by SSP and SSCP respectively, in women experiencing RSA and controls. RESULTS: The KIR repertoire did not differ between RSA patients and controls in terms of: (i) the number of inhibitory receptors; (ii) the number of activating receptors; (iii) the ratio of inhibitory to activating receptors. KIR2DL4, a receptor for HLA-G, has different transmembrane alleles, which produce functionally different phenotypes. The frequency of KIR2DL4 transmembrane genotypes differed significantly between RSA patients and controls (P=0.03). However, although homozygosity for a membrane-bound receptor was more frequent in patients (25%) than controls (10%), other genotypes that would produce the same phenotype were not more frequent in patients than controls. CONCLUSIONS: The data provide little evidence that KIR polymorphism plays a role in predisposition to RSA.

Human Pathology (33)


http://www.sciencedirect.com/science/article/B6WGD-49BXWP3-M/2/e04d91904532a5828e14d21f8531668

Balanced translocations are rare in myelodysplasia (MDS) and acute myeloid leukemia (AML) with multilineage dysplasia; however, the t(3;5)(q25;q35) and insertion variant occur in a subset of patients. To evaluate the possible genes involved in this translocation, we studied 6 cases with a t(3;5) by fluorescence in situ hybridization with probes directed against the nucleophosmin (NPM), EV11, and Ribophorin genes, as well as a newly developed myeloid leukemia factor 1 (MLF1) BAC clone. The histologic spectrum of the cases was variable, ranging from refractory cytopenia with multilineage dysplasia to AML with multilineage dysplasia in the World Health Organization classification. An NPM/MLF1 fusion was identified in 5 of 6 cases, whereas the EV11 and Ribophorin genes were not involved in any of the cases. The NPM/MLF1-positive cases were predominantly young adult males (median age, 33 years) who responded well to hematopoietic stem cell transplantation. These findings suggest that an NPM/MLF1 fusion is the primary molecular abnormality in t(3;5) MDS and AML with multilineage dysplasia, and also that cases with NPM/MLF1 may be clinically distinct from other MDS-associated disease.


http://www.sciencedirect.com/science/article/B6WGD-49KGPM6-K/2/511061193133b299967b41fb4fdd8db2

The histological transformation from a follicular lymphoma (FL) to a diffuse large B-cell lymphoma (DLBL) occurs in 22% to 30% of all cases of FL. The aim of this study was to identify specific chromosomal gains/losses associated with transformation of FL to DLBL, in addition to the well-
known mechanisms like p53 mutation and protein expression and c-myc translocation and up-regulation. This is the first study to meet 2 important conditions for such a comparison. First, we demonstrate that the FL and the DLBL were clonally related, based on identical immunoglobulin gene rearrangements in 5 of the 6 cases. Second, we used laser microdissection microscopy to isolate only the neoplastic cells from the initial FL samples. The results indicate that no single chromosomal abnormality seems to be responsible for the transformation of FL to DLBL. P53 protein overexpression was found in 4 and c-myc translocation in 3 of the 6 transformed DLBLs, but not in the initial FL samples. Additional chromosomal abnormalities were detected by comparative genomic hybridization in all 6 cases when the DLBL was compared with the FL. In the 5 cases with transformation of grade 1 or 2 FL to DLBL, gains at chromosomes 7 (5 of 5 cases), 10p1 (3 of 5 cases), 12 (3 of 5 cases), and 20p13 (2 of 5 cases) and loss at 9q (4 of 5 cases) were the most frequently found abnormalities. A gain on chromosome 7p, in combination with a loss on 9q, was found in 4 of the 5 DLBL that transformed from FL grade 1 or 2.


http://www.sciencedirect.com/science/article/B6WGD-4CBD3N7-5/2/cf3508cfecddb475c51b299617b87cd5

Microsatellite instability (MSI) is commonly seen in tumors associated with the hereditary nonpolyposis colorectal cancer syndrome and is caused by defects in the DNA mismatch repair genes. MSI has also been observed in various sporadic cancers, including colorectal, gastric, and endometrial. The role and incidence of MSI in ovarian clear cell carcinoma remain unknown. This study was conducted to evaluate the frequency of MSI in ovarian clear cell carcinomas and to evaluate the sensitivity and specificity of immunohistochemistry in predicting mismatch-repair gene deficiency. A total of 42 ovarian clear cell carcinomas were analyzed for MSI using a panel of 5 microsatellite markers (BAT25, BAT26, D5S346, D2S123, and D17S250). Alterations in the expression of hMLH1 and hMSH2 proteins in these tumors were examined. Of the 42 ovarian clear cell tumors analyzed, 6 demonstrated a high level of MSI (MSI-H), 3 demonstrated a low level of MSI (MSI-L), and the remaining 33 exhibited microsatellite stability (MSS). No correlation was found between MSI level and patient age or tumor stage or size (P >0.05). Loss of expression of either hMLH1 or hMSH2 was observed in 4 of the 6 (67.7%) MSI-H tumors, whereas 34 of the 36 (94.4%) MSI-L or MSS tumors expressed both the hMLH1 and hMSH2 gene products. Our results indicate that MSI-H is involved in the development of a subset of ovarian clear cell carcinomas. A strong correlation exists between alterations in the expression of hMLH1 and hMSH2 and the presence of MSI-H in these tumors. However, immunohistochemical testing alone may miss a small fraction of cases with MSI-H.


http://www.sciencedirect.com/science/article/B6WGD-4C4D7KX-P/2/1ad096a2216b859bd7676eeb10902ddc

Two cases of placental transmogrification of the lung are reported. The lesions presented in the left lung, in one case as a giant bulla of the upper lobe and in the other as a cystic nodule of the lower lobe. A segmentectomy was performed in both cases, and the patients were alive and well 5 years and 2 months after surgery, respectively. In our opinion, pulmonary placental transmogrification is not a variant of emphysema, as generally considered, but rather probably represents a benign proliferation of immature interstitial clear cells with secondary cystic change.
This report presents a histological, immunohistochemical, ultrastructural and molecular study of these peculiar cells, together with a review of the literature.


http://www.sciencedirect.com/science/article/B6WGD-4CBD3N7-B/2/e1df38cd47c364b64d8d395865d84686

The monitoring of gastric lymphomas is often hampered by the inherently limited sampling provided by small endoscopic biopsy specimens. To investigate the feasibility of using gastric washing fluid for monitoring patients with known gastric lymphoma and for diagnosing gastric involvement in patients with extranodal nongastric lymphoma, we collected 49 gastric washings from 39 patients (29 patients with gastric lymphoma and 10 patients with nongastric extranodal lymphoma). Collection was done at the time of follow-up biopsy and when no endoscopic abnormalities were found. DNA was extracted from the washing fluid and analyzed for clonal IgH gene rearrangement by Southern blotting (J6 probe) and/or polymerase chain reaction (PCR) (using VH-FR3 and JH primers). Forty-one of 49 samples (84%) yielded sufficient DNA for molecular analysis. Sixteen of 41 analyzable gastric washing samples (39%) failed Southern blot analysis due to degraded or insufficient DNA. Concordance between the results of Southern blot analysis of the washing and histology of the simultaneous biopsy specimen was found in 20 (80%) of the remaining 25 samples. The IgH PCR result was concordant with biopsy histology in 33 out of 41 washing samples (80%). The overall concordance between molecular clonality studies of washings (Southern blotting and/or PCR) and biopsy histology was 83% (34 of 41). Of the 7 (18%) discrepant specimens, 2 were diagnosed histologically as lymphoma, but the simultaneous washings were negative by molecular studies. Five biopsy specimens were histologically benign, but the corresponding washings demonstrated clonal IgH gene rearrangement (3 cases by PCR and 2 cases by Southern blotting). This study demonstrates the diagnostic utility of molecular clonality analysis of gastric washings.


http://www.sciencedirect.com/science/article/B6WGD-4CTDD8S-14/2/eb2c1ebd05b442b357549ce9e6c112bb

High-grade astrocytomas are tumors that are uncommon in children. Relatively few studies have been performed on their molecular properties and so it is not certain whether they follow different genetic pathways from those described in adult diffuse astrocytomas. In this study, we evaluated 24 pediatric high-grade astrocytomas (11 anaplastic astrocytomas and 13 glioblastomas) all of which were sporadic and primary. We studied mutations of p53, phosphatase and tensin homolog (PTEN), loss of heterozygosity (LOH) of chromosomes 17p13, 9p21 and 10q23-25, amplification of epidermal growth factor receptor (EGFR), and overexpression of EGFR and p53 protein. In addition, we searched for microsatellite instability (MSI) by using MSI sensitive and specific microsatellite markers. p53 mutations were found in 38% (9/24) of the high-grade astrocytomas and all brain stem tumors except 2 (71%, 5/7) had p53 mutations. PTEN mutations were found in 8% (2/24) of high-grade astrocytomas. However, no EGFR amplification was found in any of them. LOH was found at 17p13.1 in 50% (3/6 informative tumors), 9p21 in 83% (5/6 informative tumors), and 10q23-25 in 78% (7/9 informative tumors). Four tumors showed MSI, and 2 of them that showed widespread MSI were regarded as tumors with replication error (RER+) phenotype. All 4 tumors with MSI showed concurrent LOH of 9p21 and 10q23-25. Combining gene alterations, LOH, MSI, and gene mutations, inactivation of both alleles of PTEN and p53 was
found in 57% (4/7 informative tumors) and 50% (3/6 informative tumors) of the cases respectively. We conclude that development of pediatric high-grade astrocytomas may follow pathways different from the primary or secondary paradigm of adult glioblastomas. In a subset of these tumors, genomic instability was also implicated.


Chronic hepatitis may progress to cirrhosis and hepatocellular carcinoma (HCC). Progressive accumulation of mutations and genomic instability in chronic viral hepatitis might flag an increased risk of HCC development. Genomic instability at dinucleotide microsatellite loci in chromosomes 2, 13, and 17 and at 2 mononucleotide repeat loci was examined in liver tissues from 41 patients, including 30 without HCC (18 patients with chronic hepatitis and 12 with cirrhosis) and 11 with HCC. Genomic instability was detected in 51% of the 41 cases. Allelic imbalance at informative dinucleotide loci occurred in 37% of the cases. In 14 cases (34%), allelic imbalance was detected in chronic hepatitis or cirrhosis without HCC. Allelic imbalance at the chromosome 13 locus was detected in 50% of the cases of chronic hepatitis C. Allelic imbalance at the TP53 chromosome locus and/or at the chromosome 13 locus was significantly more frequent than alterations at the chromosome 2 locus (P = .026). Low-level microsatellite instability was found in 20% of all cases examined and high-level microsatellite instability in 3 patients (7.5%), including 2 cases of chronic hepatitis and 1 case of cirrhosis. Our results show that allelic imbalance occurs frequently in hepatitis-related HCC as well as in chronic hepatitis in patients without HCC. Allelic imbalance at the D13S170 chromosome 13 locus (13q31.2) occurs frequently in chronic hepatitis, suggesting that genomic alterations affecting the long arm of chromosome 13 might be used to monitor the natural progression of chronic hepatitis-associated liver carcinogenesis. HP32:698-703. Copyright (c) 2001 by W.B. Saunders Company


http://www.sciencedirect.com/science/article/B6WGD-4D04Y4P-G/2/8f200c16989f986594b17b4fe7f1c5

Disseminated disease is very important in the clinical assessment of pediatric sarcomas. Several reports suggest that reverse transcriptase polymerase chain reaction (RT-PCR) holds great promise in the early staging of cancer patients in general. However, the complexities of these protocols hamper adequate standardization, and their application as routine diagnostic tools has been difficult. The aim of this study is to assess the actual minimal number of tumor cells that may be detected by RT-PCR in a blood sample. Specific tumor cell dilutions from an Ewing's sarcoma cell line reconstituted in peripheral blood from healthy individuals were "ficolled" and submitted to RNA extraction for cDNA preparation and PCR amplification of the t(11-22) (q24;q12) fusion transcript. After PCR amplification, we were able to detect the EWS/FLI-1 chimeric gene product at a dilution of 10 tumor cells per 1 or 2 mL of blood. Our simple method supports a role for routine clinical use of RT-PCR in the detection of circulating Ewing's sarcoma cells.

http://www.sciencedirect.com/science/article/B6WGD-4C4D7KXC/2/8ebb1a490b97325f37f0ae2172f67aab

A high incidence of skin cancers has been noted around the Semipalatinsk Nuclear Testing Site (SNTS) in Kazakhstan. Recently, basal cell carcinoma (BCC) susceptibility genes, human homolog of the Drosophila pathed gene (PTCH), and the xeroderma pigmentosa group A-complementing gene (XPA), have been cloned and localized on chromosome 9q22.3. To clarify the effect of low-dose irradiation on the occurrence of BCC, we used microdissection and polymerase chain reaction to identify loss of heterozygosity (LOH) at 9q22.3 using BCC samples obtained from this region. Ten Japanese samples were analyzed as controls. LOH with at least 1 marker was identified in 5 of 14 cases from around SNTS, whereas only 1 case with 1 marker was identified among the 10 Nagasaki cases. The total number of LOH alleles from SNTS (8 of 45) was significantly higher than the number from Nagasaki (1 of 26) (P = 0.03). The higher incidence of LOH on 9q22.3 in BCC from around SNTS suggests involvement of chronic low-dose irradiation by fallout from the test site as a factor in the cancers.


http://www.sciencedirect.com/science/article/B6WGD-4FM0J29-H/2/22603702102f4c2d65dc570d69f756b5

SummaryThis report describes a vasculitis and subsequently developing angiodestructive lymphoma in an 11-year-old Japanese-Filipino girl exhibiting mosquito allergy with the background of chronic active Epstein-Barr virus (EBV) infection. She developed necrotic skin ulcer at the site of mosquito bite, and histopathological examination revealed EBV-positive mononuclear cell infiltration throughout the wall of small-sized muscular artery. These EBV-positive lymphoid cells were oligoclonal in Southern blot analysis for EBV terminal repeats. Effectiveness of steroid therapy also supports the nonneoplastic nature. Approximately 1 year later, she developed progressive large skin ulcer without mosquito bites. Microscopically, the angiocentric or angiodestructive pattern of EBV-positive atypical cells supported the diagnosis of extranodal natural killer/T-cell lymphoma. Southern blot analysis revealed the monoclonal neoplastic nature of EBV-positive cells. In contrast to the primary mosquito bite lesion, natural killer/T-cell lymphoma cells exhibited the higher expression of EBV latent membrane protein 1 mRNA and the apparent protein expression detected by immunohistochemistry.


http://www.sciencedirect.com/science/article/B6WGD-4CJ3X3S-B/2/6f673519d5374aa8be3c348f1ef07887

Gliomatosis peritonei, a rare condition that occurs almost exclusively in the setting of ovarian immature teratoma, is characterized by the occurrence of nodules of mature glial tissues in the peritoneum. It is controversial whether glial tissues are derived from maturation of the associated
teratomatous tissue that has implanted in the peritoneum, or glial differentiation of subperitoneal stem cells. In this study, we employed the unique genetic characteristics of ovarian teratomas (often with a duplicated set of maternal chromosomes and thus homozygous at many polymorphic microsatellite loci) versus normal tissues (heterozygous pattern due to presence of maternal and paternal genetic materials) to investigate the origin of gliomatosis peritonei. DNA samples were extracted from microdissected paraffin-embedded tissues, including the glial implants, the associated ovarian teratomas, and normal tissues, to determine their patterns of microsatellite loci in a multiplex polymerase chain reaction system. Two cases were not informative because the ovarian teratoma showed a heterozygous microsatellite pattern. In the 5 informative cases, the normal tissues showed a heterozygous pattern in the microsatellite loci, the associated teratomas showed a homozygous pattern, and the glial tissues showed a heterozygous pattern. Thus, gliomatosis peritonei is genetically unrelated to the associated teratoma but is probably derived from nonteratomatous cells, such as through metaplasia of submesothelial cells.


http://www.sciencedirect.com/science/article/B6WGD-4B0NG2V-R/2/2aa43e69b2e28f0e3c3c4c3ac266bc388b7

Liposclerosing myxofibrous tumor (LSMFT) is a benign fibro-osseous lesion that is characterized by mixture of histologic elements including lipoma, fibroxanthoma, myxoma, ischemic ossification, and fibrous dysplasia (FD)-like features. These tissue components are seen in the original reports of FD; however, the relationship between LSMFT and FD is not clear. Point mutation of the [alpha] subunit of G protein (Gs [alpha]), which increases cyclic adenosine monophosphate formation, has been recognized as the cause of McCune-Albright syndrome as well as polyostotic and monostotic FD of bone. Gs [alpha] mutation at the Arg201 codon in 2 patients of LSMFT was demonstrated in the present study. Although direct sequencing analysis using the fresh-frozen materials could not detect the mutation, the polymerase chain reaction fragmentation length polymorphism (PCR-RFLP) disclosed the missense point mutation Gs [alpha] at the Arg201 codon in 2 cases involving LSMFT. This result strongly suggests that a subset of LSMFT is a variant form of FD.


http://www.sciencedirect.com/science/article/B6WGD-4D04T5C-26/2/965082c4b4fdec46bf9fd84888ec6def

Bartonella (formerly Rochalimaea) henselae (Bh) plays a central role in cat scratch disease. A polymerase chain reaction (PCR)-based assay that can detect Bh DNA in formalin-fixed, paraffin-embedded (FF-PE) samples would have utility in the evaluation of processed lymph nodes suggestive of this disorder. Fresh or FF-PE cultures of Bh and related species were analyzed. Thirteen lymph nodes (12 FF-PE and one fresh cell suspension) with necrotizing suppurative granulomatous inflammation and seven FF-PE negative control lymph nodes were also evaluated. PCR was performed using a novel, heminested protocol. Amplified products were analyzed by gel electrophoresis. The fresh and FF-PE Bh cultures showed a specific PCR product with an analytical sensitivity of 0.5 pg bacterial DNA. Seven (54%) of 13 clinical lymph node samples with morphological features suggestive of cat scratch disease also had detectable Bh DNA, whereas none of the seven negative control lymph nodes yielded positive results. We
have designed a rapid and sensitive PCR test that can reliably detect Bh DNA in fresh and FF-PE samples. Our findings indicate that this assay has clinical utility in the diagnosis of cat scratch disease.


http://www.sciencedirect.com/science/article/B6WGD-4B0NG2V-M/2/b08c1f3cf6d8b2dbf4445b6bdeb370ce

Combined small cell and non--small cell carcinoma is relatively rare in the lung. Examination of the clonal relationship of different components in this type of tumor may give a clue to the rarity. We retrieved 6 such tumors; all 6 had small cell carcinoma and adenocarcinoma components, and 3 had an additional squamous cell carcinoma component. We examined the point mutations in the p53 gene and allelic loss (ie, the loss of heterozygosity [LOH] pattern) of chromosome 3p in each component. p53 mutations were detected in the small cell carcinoma component of 5 tumors and in the non--small cell carcinoma components of 2 tumors. In 1 case, the squamous cell carcinoma component had a p53 mutation locus identical to that in the small cell carcinoma component, but in the other case, the adenocarcinoma component had a different mutation than that in the small cell carcinoma component. Chromosome 3p LOH loci in the squamous cell carcinoma component were present in the small cell carcinoma component in all 3 cases, but some LOH loci were not identical in the small cell carcinoma and adenocarcinoma components in 3 cases. These results suggest that the small cell and squamous cell carcinoma components of combined small cell lung carcinomas have an intimate clonal relationship. On the other hand, the adenocarcinoma component often may be derived from a separate clone or, more likely, undergo a progressive process separate from the squamous cell--small cell carcinoma beginning in a very early stage, that is, before the appearance of p53 and chromosome 3p abnormalities. This tumorigenesis process may explain the relative rarity of combined small cell and non--small cell carcinoma, which occurs primarily in the peripheral lung, an infrequent site of squamous cell carcinoma.


http://www.sciencedirect.com/science/article/B6WGD-4CTDD8S-1J/2/dff15c492129a0434bfffbe41056b8f0

A subset of patients with non-Hodgkin's lymphoma (NHL), present with or subsequently develop lymphocytic effusions. Differential diagnosis between reactive lymphocytosis and recurrent low-grade NHL is difficult by cytology alone. We studied the use of polymerase chain reaction (PCR)-based techniques to detect concurrent/recurrent NHL. Both primary tumors and atypical lymphocytic effusions of 12 low-grade B-NHL patients and 4 T-NHL patients were studied. Six pleural effusions (reactive/carcinomatous), in patients with no history of NHL, were included. Samples were amplified by PCR, using Fr3, Fr2, LJH, and VLJH primers specific for the immunoglobulin heavy chain (IgH) gene and V[gamma]-8, V[gamma]9, V[gamma]10, V[gamma]11 and J[gamma]1/J[gamma]2 consensus primers specific for the T-cell receptor gamma (TCR-[gamma]) gene. IgH gene PCR products were analyzed by polyacrylamide gel electrophoresis (PAGE). TCR-[gamma] gene PCR products were analyzed using a novel nonradioactive single-stranded conformational polymorphism (SSCP) procedure. IgH gene rearrangement analysis demonstrated monoclonality in 11/12 primary low-grade B-NHLs. Identical monoclonal bands were found in both primary tumor and effusion in 9 patients. TCR-[gamma] gene rearrangement analysis demonstrated monoclonality in 4 of 4 primary T-NHLs. Identical
monoclonal banded patterns were found in both primary tumor and effusion in 3 patients. Our results strongly support the diagnosis of concurrent/recurrent NHL in 13 of 16 (81%) cases of atypical lymphocytic effusions. IgH/PAGE and TCR-[gamma]/SSCP analyses are useful tools in the diagnoses of lymphocytic effusions in patients with NHL.


http://www.sciencedirect.com/science/article/B6WGD-4C2RX5R-P1/2/0db58deb0fa373c95cd66fa98ee8dd673

Placental chronic villitis was observed in 44 cases (2.12%) of 2,073 histologically examined placentas. Infiltrating lymphocytes in chronic villitis were determined by immunohistochemistry to be predominantly helper/inducer T cells. Detection of the cytomegalovirus (CMV) gene was performed on paraffin-embedded sections by polymerase chain reaction (PCR) using two different primers (CMV immediate early gene and CMV late antigen gp 64). Both CMV immediate early gene and late antigen gp 64 gene were detected in one case. Cytomegalovirus late antigen gp 64 gene was observed in only three cases. Among these four cases, the cytomegalic inclusion body was observed only in a single case with light microscopic examination. In two cases generalized CMV infection was manifested during the early infantile period and the patient died of the disease. The other two cases were asymptomatic. Our data suggest that approximately 9% of the cases of chronic villitis are caused by CMV infection, and most of them are difficult to detect morphologically. Detection of CMV gene by PCR using primers, especially late antigen gp 64 gene, is very useful for assessing the cause of placental chronic villitis.


http://www.sciencedirect.com/science/article/B6WGD-48VN45R-V/2/4631384a92efed4e230d66b7986b7f5

Cutaneous lymphoid hyperplasia (CLH) has been proposed to be the benign end of a continuum of lymphoproliferative disorders with cutaneous lymphoma at its malignant extreme. An intermediate condition, known as "clonal CLH," was first recognized by us and shown to be a transitional state capable of eventuating in overt lymphoma. To better determine the prevalence of dominant clonality and risk of lymphoma among CLH cases, we studied the immunohistology and clonality of fresh-frozen samples from 44 CLH patients referred to a multidisciplinary lymphoproliferative disorders program. Using a large panel of lymphoid markers, the cases were divided into 38 typical mixed B-cell/T-cell type CLH and 6 T-cell-rich type (T-CLH), the latter containing > 90% T cells. Of the 44 patients, 38 had solitary or localized lesions (4 cases of T-CLH), and 6 had regional/generalized lesions (2 cases of T-CLH). Forty cases were of idiopathic etiology. Suspected etiologies among 4 other cases included mercuric tattoo pigment, doxepin, clozapine, and bacterial infection. Immunoglobulin heavy chain (IgH) and T-cell receptor (TCR)-gamma gene rearrangements (GR) were studied using polymerase chain reaction assays, which are approximately 80% sensitive. Overall, 27 cases (61%) showed clonal CLH: 12 IgH+ (27%; 3 cases of T-CLH); 13 TCR+ (30%; 1 case of T-CLH); and 2 IgH+/TCR+ (4%; neither case was T-CLH). Two cases (4%; 1 case of T-CLH) progressed to cutaneous B-cell lymphoma. Both of these patients presented with regional lesions. Our findings indicate that clonal overgrowth is common in CLH, links CLH to lymphoma, and probably involves both B- and T-cell lineages (although TCR GR by B cells and vice versa could not be ruled out). The high prevalence of dominant clonality in our series may have resulted from the sensitivity of our PCR assays as well as patient selection.
Strong epidemiological evidence links human papilloma viruses (HPV) with the development of cervical intraepithelial neoplasia (CIN) and invasive cancers of the uterine cervix. The localization of HPV DNA sequences high up in the female genital tract (in benign and malignant lesions) is not that uncommon, but its precise significance is uncertain. In particular, the detection of HPV DNA sequences by polymerase chain reaction (PCR) needs careful interpretation, because the source of the amplicon may emanate from tumor cells, direct contamination from the cervix, or possibly from extratumoral sites in the endometrium. We have previously reported the identification of koilocyte-like changes in the squamous epithelium of some endometrial adenoacanthomas. Adenoacanthomas (adenocarcinoma with squamous metaplasia) are mixed epithelial tumors arising in the endometrium composed of malignant glandular areas admixed with benign metaplastic squamous epithelium. The rarer adenosquamous carcinoma containing both malignant glandular and squamous areas is also described. The origin of benign/malignant squamous epithelial islands in endometrial tumors has been the subject of speculation, with some investigators considering an origin from metaplastic glandular endometrial cells. In this study, we examined 10 normal endometrial samples, 20 adenocarcinomas, 41 adenocarcinomas with squamous metaplasia, and two adenosquamous carcinomas, (including control cervical material where possible) for the presence of HPV DNA sequences using nonisotopic in situ hybridization (NISH), type-specific HPV PCR, general primer PCR (to detect sequenced and unsequenced HPVs), and PCR in situ hybridization (PCR-ISH). We did not identify HPV DNA sequences in normal endometrial tissue. In adenocarcinomas (endometrioid type), HPV was only identified in 2 of 20 cases by PCR, both of which were HPV 11 positive. We were unsuccessful in identifying HPV in endometrial carcinomas by NISH or by PCR-ISH, raising the possibility of contamination from the cervix in the two positive cases. In adenoacanthomas, a low-risk HPV type (HPV 6) was found in 19 of 41 cases. NISH signals were intranuclear in location in squamous regions of adenoacanthomas. Additional positive nuclei were uncovered using PCR-ISH, which increases the sensitivity of standard NISH detection. HPV DNA sequences were located in some malignant endometrial glandular epithelial cells, but this accounted for a minority of samples. HPV DNA sequences were not detected in extraepithelial sites. Mixed infection by two different HPV types was identified in two cases. Most cases showed similar HPV types in cervical and endometrial lesions, although discordant cases were uncovered. In adenosquamous carcinomas, one case showed mixed infection with HPV 6 and 33 by PCR. The apparent segregation of low-risk HPV type (HPV 6) with benign squamous metaplastic epithelium in adenocarcinoma with squamous metaplasia, and high-risk type (HPV 33) with malignant squamous epithelium in adenosquamous carcinoma, raises important questions in relation to the role of HPVs in mixed epithelial tumors of the endometrium and their interplay in the pathogenesis of squamous metaplasia at extracervical sites.
changes underlying the progression from APA to adenocarcinoma are unknown. DNA from paraffin-embedded tissue of 6 APAs was evaluated for microsatellite instability (MI), MLH-1 promoter hypermethylation, and CTNNB-1 mutations. Tissue sections were also subjected to MLH-1, MSH-2, and [beta]-catenin immunostaining. MI was not detected in any case. Two tumors exhibited MLH-1 promoter hypermethylation and showed focal negative MHL-1 immunostaining; 1 of these showed marked architectural complexity and cellular pleomorphism. Five cases presented [beta]-catenin nuclear immunoreactivity, but none of them had CTNNB-1 mutations. The results of this study suggest that APA and complex endometrial hyperplasia may share some molecular alterations. Some APAs exhibit MLH-1 promoter hypermethylation with focal lack of MLH-1 immunostaining, a molecular abnormality involved in the transition from complex atypical hyperplasia to endometrioid adenocarcinoma.


http://www.sciencedirect.com/science/article/B6WGD-4F4H9PP-1/2/57d5fec64763145ee6f678397b4ef2c1

SummarySupratentorial primitive neuroectodermal tumors (SPNETs) and medulloblastomas (MBs) are histologically similar intracranial tumors found in different anatomic locations of the brain. Our group has previously demonstrated that loss of chromosome 8p is a frequent event in MBs. The aim of this study was to evaluate whether DLC-1, a newly identified tumor-suppressor gene on chromosome 8p22, is involved in the tumorigenesis of MBs and the histologically similar SPNETs. We first assessed for alterations of gene expression in microdissected tumors and detected lack of DLC-1 transcript in 1 of 9 MBs (case M44) and 1 of 3 SPNETs (case M1). Neither somatic base substitutions nor homozgous deletion were found in tumors without DLC-1 transcript. We then explored the possibility of hypermethylation of the CpG island in DLC-1 as the mechanism of suppressed expression. Methylation-specific polymerase chain reaction revealed promoter hypermethylation of DLC-1 in M1 but not in M44. Bisulfite sequencing further verified a densely methylated pattern of 35 CpG sites studied in M1 that were not found in normal brain, indicating that inactivation of DLC-1 by hypermethylation is involved in SPNET. Based on this finding, we examined an additional 20 MBs, 8 SPNETs, and 4 MB and 2 SPNET cell lines for hypermethylation of the CpG island of DLC-1, finding that none of these samples exhibited DLC-1 methylation. In conclusion, our results demonstrate that transcriptional silencing of DLC-1 through promoter hypermethylation may contribute to tumorigenesis in a subset of SPNETs, and that loss of DLC-1 expression in MBs may be related to mechanisms other than promoter hypermethylation, genomic deletion, and mutation.


http://www.sciencedirect.com/science/article/B6WGD-4BHSXSD-H/2/1a8e53a77a91483359df5f2ed1401261

Previous studies have established that expression of plakoglobin is down-regulated during malignant transformation. The aim of this study was to evaluate for the first time the expression of plakoglobin at the mRNA and protein levels in primary oropharyngeal squamous cell carcinomas (SCCs) and determine the extent to which the patterns of expression correlated with clinical parameters. Plakoglobin expression was evaluated in 37 new tumor cases and normal oral epithelium using immunofluorescence, reverse transcriptase-polymerase chain reaction (RT-PCR), and Northern and Western blotting analysis. The results indicated that the steady-state
levels of plakoglobin protein were down-regulated in all tumors compared with normal epithelium. Furthermore, in 87.1% of the tumors, plakoglobin immunoreactivity displayed an abnormal cytoplasmic localization that was inversely correlated with tumor size and directly correlated with a poor clinical outcome for the patient. Northern blotting analysis revealed that down-regulation of mRNA expression occurred in only 65.6% of the tumors, with plakoglobin mRNA levels similar to normal epithelium in the remaining cases. In the tumors expressing mRNA levels similar to those of normal tissue, a 3.7-kb transcript was detected in addition to the expected 3.4-kb transcript observed in normal epithelium. RT-PCR analysis of the 3' untranslated region of the 3.7-kb plakoglobin mRNA transcript identified a 297-base insertion from +2369 to +2666 that had been previously reported only in transformed cell lines (GenBank M23410). Interestingly, the prognosis was poor for patients with tumors expressing both RNA transcripts. These results are consistent with the concept that complex regulation of plakoglobin expression and intracellular routing may contribute to malignant transformation. The study also shows evidence that the level of expression and intracellular localization of plakoglobin may be useful in predicting the course of disease in patients with oropharyngeal SCC.


http://www.sciencedirect.com/science/article/B6WGD-4D04NX7-6/2/4f6dc50298ee0842f671ad900f0c0ce50

Renal angiomyolipomas are benign tumors composed of variable amounts of mature fat, smooth muscle, and thick-walled blood vessels. They occur either sporadically or in association with tuberous sclerosis. Such tumors are considered as hamartomas, but few data are available concerning their pathogenesis. Indeed, it is not known whether angiomyolipoma is a congenital malformation or a neoplastic process. To answer this question, we assessed the clonality of sporadic angiomyolipomas using molecular analysis. Seven women (mean age, 59 years) with renal angiomyolipomas were included. DNA of the tumor and the normal adjacent kidney was extracted from archival paraffin-embedded tissue. DNA methylation pattern at a polymorphic site on the HUMARA gene was studied by polymerase chain reaction (PCR) amplification after methylation-sensitive enzyme digestion. This procedure enables the differentiation between polyclonal and monoclonal lesions according to their X-chromosome inactivation pattern. Five of the seven women included were informative for the HUMARA gene. The mean size of the angiomyolipomas was 53 mm (range, 18 to 110). In one case, a tumor thrombus was observed in the inferior vena cava. Clonal analysis showed that all of the angiomyolipomas and the tumor thrombus studied were monoclonal. This study shows that sporadic angiomyolipomas are monoclonal lesions consistent with neoplastic disorders. This result strongly supports the hypothesis that angiomyolipomas arise from the donal proliferation of an uncommited cell, which will further evolve toward different cell types.


http://www.sciencedirect.com/science/article/B6WGD-4D0Y67R-23/2/9f774cab83337d104f2fa30b8479999ae

Epithelial tumors of the ovary are the most common ovarian tumors of adult women. They exist in several different histological patterns and exhibit varying degrees of aggressiveness. Molecular genetic studies in epithelial ovarian cancer have shown that loss of heterozygosity (LOH) for regions of chromosome 17 is a common event, probably reflecting the inactivation of one or more tumor suppressor genes present on this chromosome. We examined 87 sporadic epithelial
ovarian tumors of different grade and histological type at 16 loci on this chromosome and found that 35% of them showed LOH for chromosome 17. Of these, 84% showed LOH for all informative markers, suggesting that loss of the entire chromosome 17 homologue may have occurred. Interestingly, chromosome 17 loss was observed frequently in serous tumors (49%), was less common in endometrioid tumors (15%), and was rare in mucinous tumors (4%) (P =.01 and P =.0002, respectively). Our findings support the concept that the histological subtypes of epithelial ovarian cancer may be the result of different molecular genetic events.


http://www.sciencedirect.com/science/article/B6WGD-4D04T5C-22/2/441b6f1f2683d7881bff7d21f5776975

In 11 of 35 clinically proven cases of sarcoidosis, we detected DNA sequences coding for the mycobacterial 65-kDa antigen. In four cases, the sequences were homologous to Mycobacterium avium; seven sequences were related to other nontuberculous Mycobacteria. The insertion sequence 1110, characteristic for Mycobacterium avium, was present in three cases. The insertion sequence 6110 of the Mycobacterium tuberculosis complex (M tuberculosis, africanum, bovis, BCG) was not detectable in any of the 11 cases, ruling out the presence of members of the Mycobacterium tuberculosis complex. Therefore, it seems reasonable to speculate about a mycobacterial cause in some cases of sarcoidosis.


http://www.sciencedirect.com/science/article/B6WGD-45SJD0P-82/2/f9960598494692cc20ce9db47b8c4212

A monoclonal proliferation of germinal center cells within a lymph node follicle was incidentally discovered during the staging surgical procedures in a patient with Clark III-level cutaneous melanoma. In one of the 19 axillary lymph nodes examined, we identified a single morphologically atypical lymphoid follicle, predominantly composed of medium-sized cells and immunoreactive for B-cell antigens and for the markers of germinal center origin CD10 and bcl-6. A monoclonal rearrangement of the immunoglobulins heavy chains (IgH) was documented by polymerase chain reaction after laser capture microdissection. The cells of the aberrant follicle expressed the bcl-2 protein at higher levels than the surrounding T lymphocytes in the absence of bcl-2 gene rearrangement. We propose for this lesion the designation of incipient follicular lymphoma. The present findings also confirm the previously reported association between melanoma and lymphoproliferative disorders. HP32:1410-1413. Copyright (c) 2001 by W.B. Saunders Company


http://www.sciencedirect.com/science/article/B6WGD-4D04T0F-H/2/d68131ace8cf34ea33bad9fae22b9018

Serous surface carcinoma (SSC) is a neoplasm histologically indistinguishable from typical
serous carcinomas that arise from the ovary but has a distinct clinical presentation. It is characterized by widespread peritoneal dissemination at presentation, but the ovaries are grossly normal in size and shape. If the carcinoma involves the ovaries microscopically, the tumor is confined to the surface or is minimally invasive. The recognition of this entity is important, because in some studies it appears to have a poorer prognosis than stage-matched serous cancers of the ovary. Loss of heterozygosity (LOH) of the p53 (17p) and BRCA1 (17q) tumor suppressor genes has been frequently identified in sporadic ovarian carcinomas. Although 17p LOH is correlated with common p53 gene mutations, inactivating mutations of the BRCA1 gene are uncommon in sporadic ovarian cases. In contrast, germline BRCA1 mutations are responsible for some hereditary forms of ovarian cancer, where it has been suggested that germline BRCA1 mutations confer a more favorable prognosis. In this study, 12 sporadic SSC were assessed for the presence of allelic deletions on the p53 and BRCA1 gene loci. DNA from both tumor and normal cells was obtained for LOH studies using tissue microdissection. Polymerase chain reaction (PCR) amplification was performed with the polymorphic DNA markers TP53 (17p13.1/p53 gene) and D17S579 (17q/BRCA1 gene). LOH in the p53 and BRCA1 loci was detected in 62.5% and 66.6% of the cases, respectively. In 50% of tumors informative for both markers, it is possible that an entire chromosome may be lost. In conclusion, we have shown that LOH of the p53 and BRCA1 loci is a frequent event in sporadic SSC, similar to what has been described in the usual form of serous ovarian carcinoma. Mutational analysis will be necessary to determine the exact role of these genes in this group of tumors.


http://www.sciencedirect.com/science/article/B6WGD-4D0RMJY-13/2/3802d49462cf1b1192a9235c7f20c6ca

Dysplasia in Barrett's esophagus (BE) is a precursor to adenocarcinoma and most commonly occurs as a flat, grossly undetectable lesion. Rarely, dysplasia in BE may grow as a polypoid lesion. Most BE-associated polypoid dysplastic lesions have been referred to as "adenomas" because of their histological similarity to a colonic adenoma. BE-associated polypoid dysplastic lesions have been less well characterized than the flat type. Therefore, our aim was to characterize the clinicopathologic and molecular features of five cases of BE-associated polypoid dysplasia and to review the literature on this entity. The cases were evaluated clinically, histologically, immunostained for MIB-1 and p53, and genotyped for loss of heterozygosity (LOH) at the adenomatous polyposis coli (APC) locus. Mucosal biopsy specimens of five BE patients without dysplasia, and five BE cases with high-grade flat dysplasia, were used as controls. The study patients were all male (average age, 71 years) who presented with symptoms of gastroesophageal reflux disease. Endoscopically, all five cases had a well-defined sessile or pedunculated polypoid lesion ranging from 0.4 to 1.5 cm in size in the mid (n = 1) or distal (n = 4) esophagus and were associated with specialized-type BE (four long segment, one short segment). Histologically, the polyps consisted of intestinalized epithelium with low- and high-grade dysplasia. All five cases contained adenocarcinoma (four within the polyp, one in adjacent BE). All polyps showed increased cell proliferation in the form of surface MIB-1 staining and showed positive p53 staining. Three of three (100%) informative cases showed LOH at the APC locus in the dysplastic epithelium and in areas of adenocarcinoma. All five flat dysplasia controls also showed surface MIB-1 staining and p53 positivity, and three of three informative controls showed LOH for APC. None of the nondysplastic BE controls showed any of these findings. Three patients were treated with esophagectomy and two with polypectomy. All were alive, without metastasis, from 2 months to 6 years later. A literature review of esophageal "adenomas" uncovered 12 cases. Four of these had no clinical or pathological information, two were, in fact, gastric heterotopic lesions, one was composed entirely of intestinal-type epithelium, and five were polypoid dysplastic lesions similar to the cases described here (three male, two female; mean age, 59 years). Four of these five cases were associated with adenocarcinoma in the polyp (two
In summary, BE-associated polypoid dysplasia share similar clinical, pathological, and molecular features as flat dysplasia and are often associated with adenocarcinoma. Thus, we agree with other authors who recommend that the term adenoma, which usually carries a benign connotation, be abandoned in favor of a descriptive diagnostic term, such as "BE-associated polypoid dysplasia." BE patients with this lesion should be considered strong candidates for esophageal resection similar to lesions of this kind that occur in inflammatory bowel disease.


Endometrial carcinoma is the most common invasive malignancy of the female genital tract, and it exists as two different clinicopathologic forms: an estrogen-dependent, "usual" type and an estrogen-independent "special variant" type. Despite the frequency of endometrial cancer, little is known about the molecular genetic events that contribute to its pathogenesis. The accumulation of genetic alterations identified through the study of loss of heterozygosity (LOH), gene mutation, and gene activation in tumor DNA has been associated with the establishment and progression of a variety of human malignancies. A relatively low incidence of LOH has been reported in usual type endometrial cancers; however, special variant tumors have rarely been included in the reported studies. To understand the molecular events that contribute to both forms of endometrial cancer, 31 tumors have been surveyed for events of LOH on all chromosomes. The study groups included 18 tumors of the usual type and 13 special variant tumors. Polymorphic loci were studied by Southern blot analysis and polymerase chain reaction (PCR) of microsatellite loci. Normal tissue in each case served as a control. Both frequency and patterns of LOH differed greatly between the two tumor types. Although LOH was frequently detected in the special variant tumors, it was rare in the usual type tumors. LOH was detected in only 8 of the 18 usual tumors, with chromosomes 17, 13, and 2 being the most frequently affected (22%, 20%, and 19%, respectively). In contrast, LOH was detected in all cases of special variant tumors, with chromosomes 17p, 14, and 12 showing the highest LOH (83%, 77%, and 40%, respectively). Two cases of microsatellite instability (MI) were detected among the usual type tumors. These findings suggest that the clinicopathologic phenotypes observed in these tumor types are likely caused by different tumorigenic pathways that reflect alterations of different cancer-controlling genes.


Abnormalities in the p16INK4A, CDK4, and Rb genes, which regulate transition through G1 phase of the cell cycle, have been implicated in the progression of diverse types of cancer. To evaluate the involvement of p16INK4A, CDK4, and Rb in the tumorigenesis of meningiomas, the status of these genes or gene products were examined. The genetic alteration of the p16INK4A gene was examined by homozygous deletions and by mutation analysis. The methylation status of the p16INK4A was determined by Southern blotting. Neither homozygous deletions nor point mutations of the p16INK4A gene were observed in any of the 23 meningiomas. Partial rather than complete methylation of the p16INK4A gene at SacII or Smal sites was shown in five (23.8%) meningiomas. The methylation status of the p16INK4A gene was not consistently associated with
the expression of p16INK4A in meningiomas. These results suggest that the true relationship between methylation and expression of p16INK4A may be obscured in a complex manner by various mechanisms that regulate p16INK4A expression. Aberrant expressions of pRb and CDK4 were not observed in any of the meningiomas we examined, indicating that abnormalities of the pRb and CDK4 appear to be rare in meningiomas.

Tzen, C.-Y., Y.-W. Huang, et al. (2003). "Is atypical follicular adenoma of the thyroid a preinvasive malignancy?" Human Pathology 34(7): 666.

http://www.sciencedirect.com/science/article/B6WGD-4938JNM-B/2/70cc250c3510fde970bef9468b89ce34

Among the follicular neoplasms of the thyroid, the definition and nature of atypical adenoma have been confusing. Despite the original speculation about the biologic behavior of preinvasive malignancies, this term is currently used as an expression of uncertainty. To examine the molecular features of a typical adenoma, we analyzed the p53 genes in 2 atypical adenomas and 12 control lesions (6 typical follicular adenomas and 6 follicular carcinomas). Mutations of p53 were detected in the bizarre cells of the atypical adenomas, but not in the bland-looking follicular cells or in the control specimens. Both atypical adenomas showed an identical point mutation in codon 273 (CGT->CAT), a common mutation in various human cancers, including anaplastic carcinoma of the thyroid. This finding supports the view that atypical follicular adenoma is a precursor of thyroid anaplastic carcinoma and suggests that "atypical adenoma" should not be used to express diagnostic uncertainty about the nature of a lesion.


http://www.sciencedirect.com/science/article/B6WGD-4B0NG2V-J/2/e87c5ba3f0e6a656484edf8d3ae339b1

The vast majority of in situ breast cancers represent focal lesions all derived from a single clone and requiring local treatment alone. We focused our attention on rare cases of multicentric in situ carcinomas affecting different quadrants, which required mastectomy. Defining the origin from single- or multiple-cell clones of separate independent neoplastic foci in the breast may be of pathogenetic interest and of importance in deciding the type of therapy to be administered. We employed a molecular assay based on loss of heterozygosity (LOH) and human androgen receptor assay (HUMARA) analysis of microdissected samples from 19 mastectomies. Two or more tissue samples were obtained from 7 patients with multicentric lobular in situ carcinoma (LCIS), either classical or large-cell variety; and 12 patients with multicentric ductal in situ carcinomas (DCIS), either low-grade (7 cases) or high-grade (5 cases) variety. Separate foci of high-grade (comedonic) DCIS were found to be monoclonal in nature. On the contrary, definite evidence favoring the origin from different cell clones of separate carcinomatous foci within the same breast was obtained in 2 cases of low-grade DCIS and in 6 cases of LCIS. A genetic imbalance might be the factor favoring the development of multifocal heterogeneous foci of in situ breast cancer. Such a small subgroup of in situ cancers affecting diffusely the entire breast and originating from independent foci presents both clinical and pathogenetic interest.

Until now, no definitive molecular evidence proving or disproving a true progression from superficial to invasive bladder tumors has been reported. A total of 36 lesions from 6 patients affected by invasive bladder cancer after multiple superficial recurrences were analyzed for loss of heterozygosity on 8 loci of chromosome 9 and 2 loci of chromosome 17. In addition, the clonal composition of the tumors from two female patients was examined using the human androgen receptor assay. Our data suggest that papillary bladder lesions can and sometimes do make a true progression into invasive life-threatening tumors; however, this progression is not an invariable sequence because it was definitely proven in 2 but not confirmed in 3 of the cases we examined. HP32:468-474. Copyright (c) 2001 by W.B. Saunders Company


Polymerase chain reaction (PCR) is being increasingly used in clinical laboratories for the diagnosis of human papillomavirus. From the L1 region, there are two commonly used consensus primer systems designated CP5+/G6+ and MY09/MY11. Both detect a wide variety of human papillomaviruses (HPVs). In this investigation, the authors compared the sensitivity of these approaches with the modification of hot-start PCR on 148 neutral-buffered formaldehyde-fixed cervical biopsies classified as cervical intraepithelial neoplasia (CIN) I to III. The authors chose hot-start PCR because in a previous study it proved more sensitive than cold-start PCR. Furthermore, the authors combined GP5+/GP6+ and MY09/MY11 in a two-step amplification (nested PCR) to analyze further those cases that proved negative with either GP5+/GP6+ or MY09/MY11. The authors found that the two consensus primer systems were equally sensitive with a correlation of 98%. By using GP5+/GP6+, the authors achieved an HPV positivity rate of 95% and with MY09/MY11 94%. Nested PCR did not improve HPV positivity in the CINs included in this study.

Hypertension (2)


A common intronic polymorphism, (-1332 G/A) of the angiotensin type-2 receptor gene, located on the X-chromosome, has been reported to be functional. The aim of our study was to evaluate this polymorphism for an association with left ventricular hypertrophy. Left ventricle (LV) mass was measured in 197 patients with systemic hypertension and 60 normal volunteers using a 1.5-Tesla Philips MRI system. Genotyping was performed using a restriction enzyme digestion of an
initial 310-bp polymerase chain reaction product that included the angiotensin type-2 (-1332 G/A) locus. The mean LV mass index for the male patients was 94.3{+/-}19.6 g/m2 (n=125) and for the female patients was 71.2{+/-}12.0 g/m2 (n=72). Seventy-three (37.1%) of all patients had an elevated LV mass index, defined as the mean LV mass index for normal volunteers plus 2 SD (males 77.8{+/-}9.1 g/m2, n=30; females 61.5{+/-}7.5g/m2, n=30). Comparison of LV mass index of the A_/AA genotype (mean LV mass index=82.4{+/-}21.1 g/m2; n=123) against that of the G_/GG genotype (mean LV mass index=88.1{+/-}19.0 g/m2; n=89) as a continuous variable was significant by ANOVA (P=0.044). \( \chi^2 \) Comparison between subjects with and subjects without left ventricular hypertrophy revealed an excess of the G_/GG genotype among the group with LV hypertrophy (P=0.031). We observed an association between the angiotensin type-2 receptor (-1332 G) allele and the presence of left ventricular hypertrophy in hypertensive subjects.


http://hyper.ahajournals.org/cgi/content/abstract/41/3/414

Obesity is associated with volumetric arterial hypertension and with early increase in heart rate and decreased heart rate variability. The consequences of obesity-related hypertension on heart gene regulation are poorly known and were investigated in a model of obesity-related hypertension induced by high fat diet in dogs. When compared with control animals (n=6), a 9-week high fat diet (n=6) provoked significant weight gain and increased blood pressure load and heart rate but failed to significantly change left ventricular mass assessed by echocardiography. Subtractive hybridization of dog heart cDNA libraries were used to generate sublibraries containing differentially expressed cDNAs that were in turn spotted onto membranes to create custom microarrays. Hybridizations of these microarrays with complex probes representing mRNAs expressed in right atria and left ventricles from obese hypertensive and control dogs were performed. Thirty-eight differentially expressed genes were identified; altered expression was confirmed by Northern blot analysis in 15. In addition, real-time quantitative polymerase chain reaction confirmed differential expression for 80% of the randomly chosen tested genes. Once identified, transcripts were categorized into groups involved in metabolism, cell signaling, ionic regulation, cell proliferation, protein synthesis, and tissue remodeling. In addition, we found a set of 11 cDNAs encoding proteins with unknown functions. This study clearly shows that obesity-related hypertension, lasting for only 9 weeks, causes marked changes in gene expression in right atrium as well as the left ventricle that may contribute to early functional changes in heart function and to long-term structural changes such as left ventricular hypertrophy and remodeling.

Immunity (22)


http://www.sciencedirect.com/science/article/B6WSP-44JHVW4-B/2/8f00d1e2e67e3f3545b130c7494120a7

The major murine systemic lupus erythematosus (SLE) susceptibility locus, SLe1, corresponds to
three loci independently affecting loss of tolerance to chromatin in the NZM2410 mouse. The congenic interval corresponding to Sle1c contains Cr2, which encodes complement receptors 1 and 2 (CR1/CR2, CD35/CD21). NZM2410/NZW Cr2 exhibits a single nucleotide polymorphism that introduces a novel glycosylation site, resulting in higher molecular weight proteins. This polymorphism, located in the C3d binding domain, reduces ligand binding and receptor-mediated cell signaling. Molecular modeling based on the recently solved CR2 structure in complex with C3d reveals that this glycosylation interferes with receptor dimerization. These data demonstrate a functionally significant phenotype for the NZM2410 Cr2 allele and strongly support its role as a lupus susceptibility gene.


http://www.sciencedirect.com/science/article/B6WSP-4C6JG05-5/2/fb361484fcf85eddd74f5445ea1bef4b

Contact sensitivity responses require both effective immune sensitization following cutaneous exposure to chemical haptens and antigen-specific elicitation of inflammation upon subsequent hapten challenge. We report that antigen-independent effects of IgE antibodies can promote immune sensitization to haptens in the skin. Contact sensitivity was markedly impaired in IgE-/- mice but was restored by either transfer of sensitized cells from wild-type mice or administration of hapten-relevant IgE before sensitization. Moreover, IgE-/- mice exhibited impairment in the reduction of dendritic cell numbers in the epidermis after hapten exposure. Monomeric IgE has been reported to influence mast cell function. We observed diminished contact sensitivity in mice lacking Fc[epsi]RI or mast cells, and mRNA for several mast cell-associated genes was reduced in IgE-/- versus wild-type skin after hapten exposure. We speculate that levels of IgE normally present in mice favor immune sensitization via antigen-independent but Fc[epsi]RI-dependent effects on mast cells.


http://www.sciencedirect.com/science/article/B6WSP-41GP711-F/2/1767eb15d0316198ce63eed07e2e8460

Here we describe a family of GPI-anchored cell surface proteins that function as ligands for the mouse activating NKG2D receptor. These molecules are encoded by the retinoic acid early inducible (RAE-1) and H60 minor histocompatibility antigen genes on mouse chromosome 10 and show weak homology with MHC class I. Expression of the NKG2D ligands is low or absent on normal, adult tissues; however, they are constitutively expressed on some tumors and upregulated by retinoic acid. Ectopic expression of RAE-1 and H60 confers target susceptibility to NK cell attack. These studies identify a family of ligands for the activating NKG2D receptor on NK and T cells, which may play an important role in innate and adaptive immunity.


http://www.sciencedirect.com/science/article/B6WSP-4D34CGG-8/2/7a3abd3457de6d50c8151a5e1f3834af
Cyclophilin A (CypA/Ppia) is a peptidyl-prolyl isomerase (PPIase) that binds the immunosuppressive drug cyclosporine. The resulting complex blocks T cell function by inhibiting the calcium-dependent phosphatase calcineurin. To identify the native function of CypA, long suspected of regulating signal transduction, we generated mice lacking the Ppia gene. These animals develop allergic disease, with elevated IgE and tissue infiltration by mast cells and eosinophils, that is driven by CD4+ T helper type II (Th2) cytokines. Ppia−/− Th2 cells were hypersensitive to TCR stimulation, a phenotype consistent with increased activity of Itk, a Tec family tyrosine kinase crucial for Th2 responses. CypA bound Itk via the PPIase active site. Mutation of a conformationally heterogeneous proline in the SH2 domain of Itk disrupted interaction with CypA and specifically increased Th2 cytokine production from wild-type CD4+ T cells. Thus, CypA inhibits CD4+ T cell signal transduction in the absence of cyclosporine via a regulatory proline residue in Itk.


http://www.sciencedirect.com/science/article/B6WSP-41BD8FH-7/2/fedeaac147760eed5a9662650fc28ad0

The 2C transgenic TCR is positively selected on Kb and is alloreactive for and negatively selected on Ld. To test an avidity model for positive selection, mice were bred to express different levels of surface Ld by varying the number of gene copies encoding [beta]2- microglobulin ([beta]2m) or Ld heavy chain. Whereas mice expressing 35% Ld ([beta]2m+- Ld+-) negatively selected the 2C TCR, mice expressing 2% Ld ([beta]2m-/- Ld+-) positively selected the 2C TCR. Furthermore, 2C cytotoxic T lymphocytes selected on 2% Ld showed peptide-specific cytolytic activity against Ld/p2Ca targets. These findings provide clear in vivo evidence that positive selection can occur on very low levels of the same class I antigen capable of negative selection when expressed at higher levels.


http://www.sciencedirect.com/science/article/B6WSP-433W6T5-7/2/93e74a015904d7bb60f1fc9a03e415b3

Homing behavior and function of autoimmune CD4+ T cells in vivo was analyzed before and during EAE, using MBP-specific T cells retrovirally engineered to express the gene of green fluorescent protein. The cells migrate from parathymic lymph nodes to blood and to the spleen. Preceding disease onset, large numbers of effector cells invade the CNS, with only negligible numbers left in the periphery. In early EAE, most (>90%) infiltrating CD4+ cells were effector cells. Migratory effector cells downregulate activation markers (CD25, OX-40) but upregulate several chemokine receptors and adsorb MHC class II on their membranes. Within the CNS, the effector cells are reactivated, with upregulated proinflammatory cytokines and downmodulated T cell receptor-associated structures, presumably reflecting autoantigen recognition in situ.

We have characterized a cytokine produced by Th2 cells, designated as IL-25. Infusion of mice with IL-25 induced IL-4, IL-5, and IL-13 gene expression. The induction of these cytokines resulted in Th2-like responses marked by increased serum IgE, IgG1, and IgA levels, blood eosinophilia, and pathological changes in the lungs and digestive tract that included eosinophilic infiltrates, increased mucus production, and epithelial cell hyperplasia/hypertrophy. In addition, our studies show that IL-25 induces Th2-type cytokine production by accessory cells that are MHC class II high, CD11c dull, and lineage-. These results suggest that IL-25, derived from Th2 T cells, is capable of amplifying allergic type inflammatory responses by its actions on other cell types.


The constitutive and cytokine-induced levels of major histocompatibility (MHC) class I expression are tightly controlled at the transcriptional level. In this study, it is shown that the cis-acting regulatory element site [alpha] of the MHC class I promoter is essential for the IFN[gamma]-induced transactivation of MHC class I gene expression through the ISRE. Moreover, it was discovered that the class II transactivator (CIITA), which is itself under the control of the IFN[gamma] induction pathway, strongly transactivates MHC class I gene expression and exerts its activity through site [alpha]. Therefore, site [alpha] is a crucial regulatory element, mediating the classic route of IFN[gamma] induction via the ISRE as well as a novel route of MHC class I transactivation involving CIITA.


We generated mice harboring germline mutations in which the enhancer element located 9 kb 3' of the immunoglobulin [kappa] light chain gene (3'E[kappa]) was replaced either by a single loxP site (3'E[kappa][Delta]) or by a neomycin resistance gene (3'E[kappa]N). Mice homozygous for the 3'E[kappa][Delta] mutation had substantially reduced numbers of [kappa]-expressing B cells and increased numbers of [lambda]-expressing B cells accompanied by decreased [kappa] versus [lambda] gene rearrangement. In these mutant mice, [kappa] expression was reduced in resting B cells, but was normal in activated B cells. The homozygous 3'E[kappa]N mutation resulted in a similar but more pronounced phenotype. Both mutations acted in cis. These studies show that the 3'E[kappa] is critical for establishing the normal [kappa]/[lambda] ratio, but is not absolutely essential for [kappa] gene rearrangement or, surprisingly, for normal [kappa] expression in activated B cells. These studies also imply the existence of additional regulatory elements that have overlapping function with the 3'E[kappa] element.

http://www.sciencedirect.com/science/article/B6WSP-41BD45H-5/2/24d7236144c8dca27e0cc878aadb682

Families of clonally expressed major histocompatibility complex (MHC) class I-specific receptors provide specificity to and regulate the function of natural killer (NK) cells. One of these receptors, mouse Ly49A, is expressed by 20% of NK cells and inhibits the killing of H-2Dd but not Db-expressing target cells. Here, we show that the trans-acting factor TCF-1 binds to two sites in the Ly49A promoter and regulates its activity. Moreover, we find that TCF-1 determines the size of the Ly49A NK cell subset in vivo in a dosage-dependent manner. We propose that clonal Ly49A acquisition during NK cell development is regulated by TCF-1.


http://www.sciencedirect.com/science/article/B6WSP-41CR78M-9/2/b83b46db39ddf29aff12ce8003d82d8

Melanoma lines MEL.A and MEL.B were derived from metastases removed from patient LB33 in 1988 and 1993, respectively. The MEL.A cells express several antigens recognized by autologous cytolytic T lymphocytes (CTL) on HLA class I molecules. The MEL.B cells have lost expression of all class I molecules except for HLA-A24. By stimulating autologous lymphocytes with MEL.B, we obtained an HLA-A24-restricted CTL clone that lysed these cells. A novel gene, PRAME, encodes the antigen. It is expressed in a large proportion of tumors and also in some normal tissues, albeit at a lower level. Surprisingly, the CTL failed to lyse MEL.A, even though these cells expressed the gene PRAME. The CTL expresses an NK inhibitory receptor that inhibits its lytic activity upon interaction with HLA-Cw7 molecules, which are present on MEL.A cells and not on MEL.B. Such CTL, active against tumor cells showing partial HLA loss, may constitute an intermediate line of anti-tumor defense between the CTL, which recognize highly specific tumor antigens, and the NK cells, which recognize HLA loss variants.


http://www.sciencedirect.com/science/article/B6WSP-41BD3W4-9/2/e2ba8a4019d9093fa2a636a2e9fa101f

Successful in-frame rearrangement of immunoglobulin heavy chain genes or T cell antigen receptor (TCR) [beta] chain genes in lymphocyte progenitors results in formation of pre-BCR and pre-TCR complexes. These complexes signal progenitor cells to mature, expand in cell number, and suppress further rearrangements at the immunoglobulin heavy chain or TCR[beta] chain loci, thereby ensuring allelic exclusion. We used transgenic expression of a constitutively active form of c-Raf-1 (Raf-CAAX) to demonstrate that activation of the Map kinase pathway can stimulate both maturation and expansion of B and T lymphocytes, even in the absence of pre-TCR or pre-BCR formation. However, the same Raf signal did not mediate allelic exclusion. We conclude that maturation of lymphocyte progenitors and allelic exclusion require distinct signals.

http://www.sciencedirect.com/science/article/B6WSP-4598W92-7/2/2f1abcfb730bed06a24ee2b59c554425

Notch1 signaling drives T cell development at the expense of B cell development from a common precursor, an effect that is dependent on a C-terminal Notch1 transcriptional activation domain. The function of Deltex1, initially identified as a positive modulator of Notch function in a genetic screen in Drosophila, is poorly understood. We now demonstrate that, in contrast to Notch1, enforced expression of Deltex1 in hematopoietic progenitors results in B cell development at the expense of T cell development in fetal thymic organ culture and in vivo. Consistent with these effects, Deltex1 antagonizes Notch1 signaling in transcriptional reporter assays by inhibiting coactivator recruitment. These data suggest that a balance of inductive Notch1 signals and inhibitory signals mediated through Deltex1 and other modulators regulate T-B lineage commitment.


http://www.sciencedirect.com/science/article/B6WSP-41BD623-4/2/1abe42d3df9e14ee5f8d891ded5315a6

The generation of an adaptive immune response against intracellular pathogens requires the recruitment of effector T cells to sites of infection. Here we show that the chemokine IP-10, a specific chemoattractant for activated T cells, controls this process in mice naturally infected with Toxoplasma gondii. Neutralization of IP-10 in infected mice inhibited the massive influx of T cells into tissues and impaired antigen-specific T cell effector functions. This resulted in >1000-fold increase in tissue parasite burden and a marked increase in mortality compared to control antibody-treated mice. These observations suggest that IP-10 may play a broader role in the localization and function of effector T cells at sites of Th1 inflammation.


http://www.sciencedirect.com/science/article/B6WSP-48M81Y9-5/2/160ca12b137662d01959cf7a243b5fdc

This study challenges the concept that herpes simplex virus type 1 (HSV-1) latency represents a silent infection that is ignored by the host immune system, and suggests antigen-directed retention of memory CD8+ T cells. CD8+ T cells specific for the immunodominant gB498-505 HSV-1 epitope are selectively retained in the ophthalmic branch of the latently infected trigeminal ganglion, where they acquire and maintain an activation phenotype and the capacity to produce IFN-γ. Some CD8+ T cells showed TCR polarization to junctions with neurons. A gB498-505 peptide-specific CD8+ T cell clone can block HSV-1 reactivation from latency in ex vivo trigeminal ganglion cultures. We conclude that CD8+ T cells provide active surveillance of HSV-1 gene expression in latently infected sensory neurons.

The degree of heavy chain (H) editing, the types of V[kappa] editors, and the pattern of J[kappa] usage are correlated with a range of the affinity of anti-DNA. This range was determined by the number and location of arginine (R) residues in the VH. We, here, changed a key arginine residue in the VH of anti-DNA transgene to glycine, which sharply reduces the affinity for dsDNA. However, complete reversion of this anti-DNA to germline enhances the affinity for phosphatidylserine (PS). The B cells of this low-affinity anti-DNA and anti-PS transgenic mouse are tightly regulated by receptor editing. Thus, anti-PS B cells are another example of a constitutive self-antigen regulated in the bone marrow.


Receptor editing is a means by which immature bone marrow B cells can become self-tolerant. Rearrangements of heavy (H) and/or light (L) chain genes are induced by encounter with autoantigens to change the specificity from self to nonself. We have developed site-directed transgenic mice (sd-tg) whose transgenes code for the H chain of antibodies that bind DNA. B cells that express the transgenic H chain associate mainly with four of the 93 functional V[kappa] genes of the mouse. Numerous aspartate residues that might inhibit DNA binding by the VH domain distinguish these L chain V[kappa] sequences, but engaging these V[kappa] editors often requires multiple rearrangements. Among the edited B cells is a subset of multispecific cells that express multiple receptors. One consequence of multispecificity is partial autoreactivity; these multispecific B cells may contribute to autoimmunity.


We have used gene-targeted mutation to assess the role of the T cell receptor [delta] (TCR[delta]) enhancer (E[delta]) in [alpha][beta] and [gamma][delta] T cell development. Mice lacking E[delta] exhibited no defects in [alpha][beta] T cell development but had a severe reduction in thymic and peripheral [gamma][delta] T cells and decreased VDJ[delta] rearrangements. Simultaneous deletion of both E[delta] and the TCR[alpha] enhancer (E[alpha]) demonstrated that residual TCR[delta] rearrangements were not driven by E[alpha], implicating additional elements in TCR[delta] locus accessibility. Surprisingly, while deletion of E[delta] severely impaired germline TCR[delta] expression in double-negative thymocytes, absence of E[delta] did not affect expression of mature [delta] transcripts in [gamma][delta] T cells. We conclude that E[delta] has an important role in TCR[delta] locus regulation at early, but not late, stages of [gamma][delta] T cell development.

We have used gene targeted mutational approaches to assess the role of the T cell receptor \([\alpha]\) (TCR[\alpha]) enhancer (E[\alpha]) in the control of TCR[\alpha] and TCR[\delta] gene rearrangement and expression. We show that E[\alpha] functions in cis to promote V[\alpha] to J[\alpha] rearrangement across the entire J[\alpha] locus, a distance of greater than 70 kb. We also show that E[\alpha] is required for normal [\alpha][\beta] T cell development; in this lineage, E[\alpha] is required for germline J[\alpha] expression, for normal expression levels of rearranged V[\alpha]J[\alpha] genes, and for expression of a diverse V[\alpha] repertoire. In [\gamma][\delta] T cells, E[\alpha] is not required for V[\delta]DJ[\delta] rearrangement, but, surprisingly, is required for normal expression levels of mature V[\delta]DJ[\delta] transcripts and for expression of germline J[\alpha] transcripts. Our findings imply that E[\alpha] function is not limited to the TCR[\alpha] components of the TCR[\alpha]/[\delta] locus or to the [\alpha][\beta] lineage; rather, E[\alpha] function is important in both [\alpha][\beta] and [\gamma][\delta] lineage T cells.


The presence and expression of killer inhibitory receptor (KIR) and CD94:NKG2 genes from 68 donors were analyzed using molecular typing techniques. The genes encoding CD94:NKG2 receptors were present in each person, but KIR gene possession varied. Most individuals expressed inhibitory KIR for the three well-defined HLA-B and -C ligands, but noninhibitory KIR genes were more variable. Twenty different KIR phenotypes were defined. Two groups of KIR haplotypes were distinguished and occurred at relatively even frequency. Group A KIR haplotypes consist of six genes: the main inhibitory KIR, one noninhibitory KIR, and a structurally divergent KIR. Allelic polymorphism within five KIR genes was detected. Group B comprises more noninhibitory KIR genes and contains at least one additional gene not represented in group A. The KIR locus therefore appears to be polygenic and polymorphic within the human population.


Somatic hypermutation (SHM) requires selective targeting of the mutational machinery to the variable region of the immunoglobulin heavy chain gene. The induction of SHM in the BL2 cell line upon costimulation is associated with hyperacetylation of the chromatin at the variable region but not at the constant region. The V region-restricted histone hyperacetylation resulting from costimulation occurs independent of AID expression and mutation. Interestingly, costimulation in the presence of Trichostatin A causes hyperacetylation of histones associated with the constant region and extends mutations to the constant region. Under this condition, promoter proximal mutations are observed in the variable region as well. The overexpression of AID results in a similar deregulation of mutational targeting. Our results indicate that the stimulation of SHM in BL2 cells activates two independent pathways resulting in histone modifications that permit induced levels of AID to selectively target the variable region for mutation.
We previously identified possible intermediates in V(D)J recombination at the TCR[delta] locus and characterized molecules with signal ends and with covalently sealed (hairpin) coding ends in thymocytes of scid mice by Southern blotting. Here, we use a sensitive ligation-mediated PCR assay to demonstrate that all coding ends detected in acid thymocytes are covalently sealed. Neither coding nor signal ends exhibit loss or addition of nucleotides. These data imply that hairpin formation is coupled to the initial cleavage at the signal/coding border, and that the cleavage step in V(D)J recombination is conservative. In scid/+ or wild-type thymocytes, hairpin coding ends are at least 1000-fold less abundant than signal ends. These results provide insight into the mechanism of V(D)J recombination.

Immuno-analyse & Biologie Specialisee (1)


ResumeLe propos de cette etude est de rapporter les performances des techniques d'amplification genique (test PCR) sur le liquide amniotique pour le diagnostic prenatal de la toxoplasmose congenitale. Une amniocentese a ete realisee chez 261 patientes atteintes de toxoplasmose pendant leur grossesse. La recherche du toxoplasme dans le liquide amniotique a ete effectuee par PCR et inoculation a la souris. Parmi les 60 cas de toxoplasmose congenitale, 56 (21 %) ont ete depistes par ce diagnostic. La sensibilite de la PCR (90 %) est superieure a celle de l'inoculation a la souris (70 %). Les deux methodes ont une specificite et une valeur predictive positive de 100 %. La valeur predictive negative est de 94 % pour la PCR et 83 % pour l'inoculation. Tous les cas diagnostiques par le bilan prenatal ont ete confirmes par le suivi serologique de l'enfant. Le test PCR pratique sur le liquide amniotique est une methode simple, rapide et d'un grand apport pour le diagnostic prenatal d'une toxoplasmose congenitale. Il permet d'instituer un diagnostic et un traitement precoce des foetus infectes. Cet examen associe aux echographies limite le nombre d'interruptions de grossesse.

Immunobiology (1)

http://www.sciencedirect.com/science/article/B7GW1-4DN9WTG-1/2/4bd44c28f7651ae6213caefc0047a877

In bitches, the onset of pyometra, an infection of the uterus, characteristically occurs in the first half of the diestrous stage in the estrous cycle, in which the blood concentration of progesterone peaks and that of estradiol-17[beta] is lowest. To investigate the immunological mechanisms governing stage-specific onset of pyometra, peripheral blood mononuclear cells (PBMNCs) were collected from beagle bitches during different stages of the estrous cycle and examined using various immunological assays. When we examined the proliferative response of PBMNCs to PYO-252, that is a clone of Escherichia coli isolated from the uterus of a dog afflicted with pyometra, the response of PBMNCs significantly decreased in the first half (day 10) of diestrus, but increased in proestrus/estrus. No significant differences were observed in the responses to concanavaline A between stages of the cycle. Throughout the estrous cycle, canine PBMNCs did not respond to lipopolysaccharide derived from *E*. coli. The response of PBMNCs collected in anestrus to PYO-252 was significantly enhanced upon the addition of estradiol-17[beta] to the culture. In contrast, these responses were significantly suppressed in the presence of progesterone. Progesterone progenitor or metabolite molecules, which have a low affinity for the progesterone receptor, did not affect proliferative responses. Expression of gamma interferon (IFN[gamma]) in response to PYO-252 was also significantly enhanced by estradiol-17[beta], but suppressed by progesterone. This evidence suggests that in the first half of the diestrous stage, suppressed activity of cellular immunity results from increasing progesterone concentration and minimal estrogen release. This marked decrease of immune resistance allows the expansion of *E*. coli, which enter the uterine cavity through the loosened cervical canal during estrus, leading to pyometra onset.

*Immunology Letters* (20)


http://www.sciencedirect.com/science/article/B6T75-4961P42-1/2/dab210f3298034837e83afc27870577d

Immune response against self antigens is normally prevented by an elaborate immunotolerance mechanism. A potential problem for recipients of gene therapy is, therefore, an immune response against the newly introduced gene product. To examine this issue we tested the immune response to the native proteins in knockout (KO) mice in which the genes for [alpha]A- or [alpha]B-crystallin were disrupted by partial or complete gene deletion, respectively. [alpha]A- and [alpha]B-crystallins are two immunologically distinct polypeptides which form the large (~800 kDa) complex in the lens referred to as [alpha]-crystallin. When immunized with murine [alpha]-crystallin, [alpha]B-crystallin KO mice, in which the corresponding gene was completely deleted, responded well to the absent self antigen. In contrast, [alpha]A-crystallin KO mice, with the partial gene deletion, resembled wild type (WT) mice in being immunotolerant toward the native crystallin. Although no functional [alpha]A-crystallin could be detected in the lens of [alpha]A-crystallin KO mice, mRNA transcript coding for a truncated [alpha]A-crystallin gene was found in thymi of these mice, suggesting that thymic expression of a residual fragment of the protein is
responsible for the tolerance induction. These data suggest that nonfunctional proteins may induce immunotolerance and protect recipients of gene therapy from immunity against the native proteins.


http://www.sciencedirect.com/science/article/B6T75-44W458H-1/2/9e6905d20ad9a06aa63c140fba57c24f

We have shown that two of the matrix metalloproteinases (MMPs), matrilysin and stromelysin-1, are capable of cleaving all of the human IgG subclasses. The cleavage occurs at a conserved site in the CH2 domain of the heavy chain of IgG, releasing a single chain Fc-like fragment. We have not been able to demonstrate cleavage of IgA, IgD, IgM or IgE classes, which lack the cleavage site, nor could we show cleavage of IgG by collagenase, gelatinase, macrophage metalloelastase or membrane-type (MT)-MMP. This cleavage of IgG, by separating the antigen-binding (Fab')2 from the Fc portion, will remove much of the immunoglobulins' functionality, e.g. complement fixation, Fc receptor binding. In the context of a tumour producing matrilysin or stromelysin, this may represent a way in which the tumour protects itself from ADCC. In inflammed or damaged tissues where plasma protein leakage occurs, degradation by MMPs may be a mechanism for clearance of IgG.


http://www.sciencedirect.com/science/article/B6T75-48GVKTX-2/2/9b4f3f8f74215690e0de7d780ac91cdf

Although [gamma][delta] T-cells form only a small portion of circulating T-cells in mice and humans, they are more frequent in many other types of mammals and this has lead to speculation regarding their roles and the evolutionary significance of their relative abundance. Moreover, whilst clear homologues of four types of T-cell receptor (TCR) chains ([alpha], [beta], [delta] and [gamma]) have been identified in vertebrates as distantly related as eutherian mammals and cartilaginous fish, there are still many gaps in our knowledge of these TCR components from various taxa. Such knowledge would further illuminate the evolution and function of these receptors and of [gamma][delta] T-cells. Here, we report the molecular cloning of a TCR-[delta] chain cDNA from the tammar wallaby (Macropus eugenii) which represents the first component of the [gamma][delta] TCR to be characterised from a marsupial. A PCR-based survey of variable (V) segment usage in tammar wallaby mammary-associated lymph node indicated that, although [gamma][delta] T-cells may be sparse in this type of tissue, this species has at least three subfamilies of V genes that have been broadly conserved across vertebrate evolution. Two V subfamilies found in the tammar wallaby were relatively similar and may have diverged more recently, an event that probably occurred at some point in the marsupial lineage.


http://www.sciencedirect.com/science/article/B6T75-44XDTFY-2/2/baf5e6cac2c6f46c38c7c1d5c82473ef
Interleukin-6, a multifunctional cytokine upon binding to its receptor on hepatocytes regulates production of acute phase proteins involved in local and systemic inflammation. Gene expression and biosynthesis of IL-6 and its receptor (IL-6 R/gp130) is under complex regulation. Histamine, in addition to its principal role in immediate type hypersensitivity has been described to modulate IL-6 production and expression of IL-6 receptor. In this study, the IL-6 and IL-6 receptor expression was examined in histamine deficient histidine decarboxylase (HDC) knock-out mouse model. Our data suggest that in histamine deficient mice the inducibility of IL-6 is significantly reduced, whilst more IL-6 receptor/gp130 mRNA expresses in the liver than in wild type (HDC+/+) mice. These in vivo findings confirm earlier in vitro results and emphasize the efficacy of antihistamines in local IL-6 related processes.


The effect of histamine and histamine antagonists was examined on gene expression and biosynthesis of bacterial endotoxin (LPS) induced interferon [gamma] (IFN[gamma]) both in human peripheral mononuclear cells (PMBC) and in T-cell enriched fractions. We found, that histamine inhibited the LPS induced transcription of IFN[gamma] gene and biosynthesis of IFN[gamma] protein in PMBC and also in CD19-depleted cell populations. The inhibitory effect of histamine could be reversed by the H2 histamine receptor (HR2) antagonists cimetidine and ranitidine both in PMBC and in CD19-depleted cells, but not with triprolidine, an H1 receptor antagonist, suggesting that the inhibition of IFN[gamma] production is mediated through H2 receptors in these cell populations. In contrast to the inhibitory effect of histamine, cimetidine alone (in the absence of exogenous histamine) strongly stimulated both the IFN[gamma] mRNA and protein production, whereas this effect was hardly seen by and other H2 receptor blocker, ranitidine. This superinduction of IFN[gamma] gene by cimetidine disappeared if the CD19+ cells are removed from PMBC. These results suggest, that inhibition of IFN[gamma] gene expression by histamine is a direct effect of histamine on H2 receptor of T lymphocytes; however, the superinduction of IFN[gamma] by cimetidine requires the presence of other (probably primarily B) cell subsets.


Although a high level of IgE is produced after primary infection with Nippostrongylus brasiliensis (Nb), most of the IgE antibodies (Abs) are not specific to the worm. Analyses with Western blotting and enzyme-linked immunosorbent assay (ELISA) revealed that the IgE Abs from Nb-infected BALB/c mice did not show reactivity with Nb-derived excretory-secretory proteins (NES) and antigens present in the cell-free extracts of the worm. Monoclonal IgE Abs obtained from the Nb-infected mice were not reactive with these Nb antigen either. To characterize Nb-induced IgE response, we used (QM x C57BL/6)F1 (QBF1) mice that bear the knock-in 17.2.25 VHDJH segment (VHT) encoding a VH region specific to 4-hydroxy-3-nitrophenylacetyl hapten, and express VHT-encoded antigen receptors on 80-85% of their B cells. Consistent with the frequency of VHT-positive B cells, more than 80% of IgE Abs induced in QBF1 B cells that were cultured with LPS plus IL-4 were found to bear VHT-encoded H chains. In contrast, when QBF1
mice were infected with Nb, less than 10% of Nb-induced IgE Abs were found to use VHT. The QBF1-derived IgE did not react with Nb antigens either. Taken together, data suggest that Nb-induced IgE response in mice is not merely the result of polyclonal activation of B cells, but may involve a mechanism that revises Ig genes secondarily.


http://www.sciencedirect.com/science/article/B6T75-4BG3SG7-3/2/3cf7ee9df076ff67fb4167d0d47e2245

Dendritic cells (DCs) are powerful antigen-presenting cells (APCs) that have attracted attention in recent years from the viewpoint of DC vaccine therapy against cancer. However, the existence of a strongly immunosuppressed state in cancer-bearing individuals inhibits DC maturation, which is one of the problems facing anti-cancer DC vaccine therapy. Protein-bound polysaccharide K (PSK), which is extracted from the cultured mycelium of Coriolus versicolor (Fr.) Quel, is used as an anti-cancer agent in Japan. PSK is reported to improve the immunosuppressed state and might be associated with DC maturation directly. We examined the effect of PSK on the maturation of DC derived from CD14-positive cells obtained from human peripheral blood monocytes using a negative selection method. CD14-positive cells cultured in the presence of PSK significantly increased the expression of HLA class II antigen and CD40; significantly increased the number and expression of CD80-, CD86- and CD83-positive cells; decreased Fluorescein isothiocyanate (FITC)-dextran uptake, augmented IL-12 production; augmented the allogeneic mixed lymphocyte reaction; and induced antigen-specific cytotoxicity. These results indicate that PSK promotes both the phenotypic and functional maturation of DC derived from human CD14-positive mononuclear cells. The clinical significance of the combined use of PSK in DC vaccine therapy remains for study.


http://www.sciencedirect.com/science/article/B6T75-45TTVW3-2/2/3816990c0eb29b6a0f075f0ec934357

In normal human subjects a small proportion of peripheral blood T-cells simultaneously express both CD4 and CD8 differentiation antigens. In this study we characterized a subset of CD4+ clones, from a healthy donor, that is specific for the thyrotropin receptor (TSHR) and that showed cells co-expressing the CD8 receptor. To address whether the expression of the CD8 receptor on the cell membrane was associated to differences in the physiology of the T-cells, we isolated, from the same clone, CD4 single positive (SP) cells from those co-expressing CD4/CD8 receptors (DP cells) and stimulated them in vitro with antigen presenting cells (APC) carrying TSHR. The results demonstrated that CD8 co-expression has a profound effect on the physiology of T helper (Th) cells. In comparison to cells expressing the CD4 receptor alone, DP T-cells showed: (1) increased proliferation; (2) higher and more sustained release of free Ca2+ in the cytosol, under stimulus; (3) lower levels of IL-2 and IL-4 released in the supernatants; (4) increased amounts of IFN-[gamma] released.

Nietfeld, W. and A. Meyerhans (1996). "Cloning and sequencing of hlk-1, a cDNA encoding a human
A population of peripheral B cells have been shown to express recombination activating gene products, RAG-1 and RAG-2, which are considered to be involved in revising the B cell antigen receptor (BCR) in the periphery. BCR engagement has been reported to turn off RAG expression in peripheral B cells, whereas the same treatment has an opposite effect on immature B cells in the bone marrow. In contrast to receptor editing that is involved in the removal of autoreactivity in immature B cells, it has been shown that secondary V(D)J rearrangement in peripheral B cells, termed receptor revision, contributes to affinity maturation of antibodies. Here, we show that RAG-2 expression in murine splenic B cells was abrogated by the coligation of BCR with complement receptors (CD21/CD35) much more efficiently than by the engagement of BCR alone. On the other hand, the same coligation augmented proliferation of anti-CD40-stimulated B cells. These findings suggest a crucial role for CD21/CD35 in directing the conservation or the revision of BCRs in peripheral B cells.


http://www.sciencedirect.com/science/article/B6T75-476VR4N-1P/2/fc368c2e5e14ded66781c9d66b1d6bc8

The T-cell receptor (TCR) can acquire a new antigen binding site by treatment with a bifunctional antibody (BFA) prepared with mAb against a specified antigen and an epitope of the TCR. Lymphocytes armed with BFA directed to CD3 and an HIV antigen were able to eliminate all HIV antigen-positive cells during incubation with a mixture of HIV-infected and uninfected cells. HIV antigen-positive cells even from persistently infected cells were undetectable with immunofluorescence staining although HIV genes were detectable by polymerase chain reaction (PCR) amplification indicating that only dormant infected or low producer cells, if any, survived. This suggests that HIV antigen-positive cells could be eliminated by administration of BFA-armed lymphocytes leaving HIV patients with only dormantly infected or low producer cells.


http://www.sciencedirect.com/science/article/B6T75-4DS3Y2G-1/2/6718787a6ac8e902cd12260bd842e826

Estrogens have been shown to modulate immune responses. Several studies have demonstrated the capacity of T cells, B cells, and monocytes to respond to estrogens and estrogen receptor...
(ER) expression in these cell types has been reported. However, little is known regarding the relative expression in these cells of ER[alpha] and the more recently identified ER[beta]. In the present study, results of quantitative TaqMan\textsuperscript{\textregistered} RT-PCR analyses indicate that ERs are differentially expressed in PBMC subsets. CD4+ T cells express relatively high levels of ER[alpha] mRNA compared with ER[beta], whereas B cells express high levels of ER[beta] mRNA but low levels of ER[alpha]. Peripheral blood CD8+ T cells and monocytes express low but comparable levels of both ERs. This quantitative analysis of ER expression in distinct PBMC subsets may provide a basis for dissecting the mechanisms of immune modulation by estrogens and identifying therapeutic targets for the treatment of inflammatory and immunologic disorders.


http://www.sciencedirect.com/science/article/B6T75-3WJ6WVJ-3/2/25b62552eaf89ba562177a853f75e2f2

Fc[gamma]RIIa is one of a family of specific cell surface receptors for immunoglobulin. Fc[gamma]RIIa, which binds immune complexes of certain IgG isotypes, plays important roles in immune homeostasis. However, the precise characteristics of IgG binding and three-dimensional structure of Fc[gamma]RIIa have not been reported. This study describes the affinity of the Fc[gamma]RIIa:IgG interaction as well as biochemical characterisation of recombinant Fc[gamma]RIIa that has been used to generate high quality crystals. Equilibrium binding analysis of the Fc[gamma]RIIa:IgG interaction found, IgG3 binds with an affinity of KD=0.6 [mu]M, as expected. Unlike other Fc[gamma]R, IgG4 also bound to Fc[gamma]RIIa, KD=3 [mu]M, clearly establishing Fc[gamma]RIIa as an IgG4 receptor. Biochemical analysis of mammalian and insect cell derived Fc[gamma]RIIa established the genuine N-terminus with Q being the first amino acid in the sequence Q, A, A, A, P. extending the N-terminus further than previously thought. Furthermore, both potential N-linked glycosylation sites are occupied. Electrospray ionisation mass spectrometry (ESMS) indicate that the N-glycans of baculovirus derived Fc[gamma]RIIa are core mannose oligosaccharide side chains. Finally, we describe the first crystallisation of diffraction quality crystals of soluble Fc[gamma]RIIa. Orthorhombic crystals diffract X-rays beyond 2.1 A resolution in the space group P21212 with cell dimensions a=78.8 A, b=100.5 A, c=27.8 A. This marks a significant advance towards understanding the three-dimensional structure of Fc[gamma]RIIa and related FcR proteins that share high amino acid identity with Fc[gamma]RIIa.


http://www.sciencedirect.com/science/article/B6T75-46MT7DR-1/2/060310fc7b16a60508e10538761fa9c7

Chondroitin sulfate (CS) is a glycosaminoglycan that is widely present in animals organisms, and it has anti-inflammatory and chondroprotective properties. To examine the effects of CS on the immune system, splenocytes obtained from ovalbumin (OVA)-sensitized BALB/c mice were challenged with OVA in the presence of CS, and cytokine levels in the medium of the cultured cells were measured. CS induced secretion of Th1-type cytokines (IFN-[gamma], IL-2, and IL-12) by OVA-sensitized splenocytes but suppressed secretion of Th2-type cytokines (IL-5 and IL-10). Flow cytometric assay showed a significantly higher percentage of helper T cells (CD4+CD8- cells) among the splenocytes cultured with OVA and CS than with OVA alone. Analysis of the IFN-[gamma] mRNA level of the splenocytes by the real-time quantitative RT-PCR technique revealed higher levels in the splenocytes cultured with OVA and CS than in the splenocytes cultured with OVA alone. This is the first demonstration that CS inhibits antigen-induced IgE
production through induction of cytokine secretion by Th1 cells, and this finding suggests a potential use of CS in preventing IgE-mediated allergy.


http://www.sciencedirect.com/science/article/B6T75-48JSXFG-2/2/5084f51bc4fcc85c130fb92577b5f56

Neurotrophins, including nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 (NT-3) are essential factors for the development of the nervous system. In this report, we demonstrate gene expression of neurotrophins and their receptors in T helper 1 (Th1) and T helper 2 (Th2) cells induced from naive CD4+CD45RB+ T cells of ovalbumin-specific DO11.10 T cell receptor transgenic mice. Interestingly, the TrkC gene, which encodes a high affinity receptor for NT-3, was expressed in Th2 cells, but not in Th1 and naive CD4+ T cells. Expression of the TrkC gene was markedly augmented by addition of anti-IFN-[gamma] monoclonal antibody (mAb) into the culture, whereas it was blocked by anti-IL-4 mAb. Moreover, NT-3 synergistically enhanced anti-CD3 mAb-induced IL-4 production by Th2 cells, but did not affect IFN-[gamma] production by Th1 cells. These data suggest that NT-3, through its receptor TrkC, plays a critical role in regulating the Th1/Th2 balance.


http://www.sciencedirect.com/science/article/B6T75-3SRRK9B-X/2/d6a8eb4f8f1620e50db8f3bac53b758d

We have analysed the relative T cell receptor (TCR) BV gene usage in T cells from hearts and spleens of CBA/HJ mice chronically infected with the Tulahuen strain of Trypanosoma cruzi. During chronic infection, CBA/HJ mice recruit T cells at the major site of inflammation (i.e. the heart), with over-representation of certain TCRBV gene subfamilies (TCRBV8S2 and TCRBV8S3). In contrast, no signal or a very weak message from a limited number of T cells was recorded from one heart of the control group. No alteration of TCRBV distribution was recorded in spleens of chronically infected CBA/HJ. Our findings indicate that there is a preferential TCRBV gene usage in the T cell response in the hearts of chronically infected mice. Furthermore, the pattern of CDR3 lengths in inflammatory T cells was altered.


http://www.sciencedirect.com/science/article/B6T75-416BXVT-6/2/6403946bf265962095931e9924adb1a1

Depletion of lymphocyte subsets in vivo using monoclonal antibodies against cell surface markers has helped to define the roles for these subsets in many immune processes. However, in some cases the mechanisms through which these lymphocytes act remain partially elucidated or completely unknown. A new approach to these biological problems is the use of transcriptional
analyses to find mRNAs whose abundance in tissues is altered by depletion of lymphocyte subsets. We have verified the use mRNA differential display (DD) for this purpose and applied it in a study of CD8+ lymphocyte mediated clearance of herpes simplex virus (HSV) from the nervous systems of experimentally infected mice. The results of the differential displays and characterisation of a large mRNA identified using this strategy are presented.


http://www.sciencedirect.com/science/article/B6T75-47RS0KB-1/2/fe79c9aa0195668d30b3f8efa1397068

Eosinophils play a pivotal role in the mechanism of allergic diseases including asthma. Interleukin-5 (IL-5) and eotaxin are critical cytokines/chemokines for eosinophil activation. Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]) is a nuclear receptor that regulates lipid metabolism. Recent evidence has suggested that PPAR[gamma] serves as a negative regulator in the immune system. In the present study, we investigated the expression of PPAR[gamma] and effect of PPAR[gamma] agonist on human eosinophils. We demonstrated that purified eosinophils and Eo-1 cells express PPAR[gamma] at the mRNA and protein levels. The PPAR[gamma] agonist troglitazone reduced the IL-5-stimulated, but not spontaneous, eosinophil survival in a concentration-dependent manner. Moreover, the eotaxin-directed eosinophil chemotaxis was dose-dependently inhibited by troglitazone. Our results suggest that the administration of the PPAR[gamma] agonists thiazolidinediones could be a new therapeutic modality for the treatment of allergic diseases such as asthma.


http://www.sciencedirect.com/science/article/B6T75-3WRB3VC-H/2/0b1c84a0c094d5f5487190b619c3ae96

The neutrophil antigen (NA)1 and 2 is coded by two recognized allelic forms of Fc gamma receptor IIIB (Fc[gamma]RIIIB). Fc[gamma]RIIIB is a low affinity receptor and preferentially removes immune complexes from the circulation. Systemic lupus erythematosus (SLE) is an autoimmune and polygenic disorder characterized by accumulation of autoimmune complexes. The majority of SLE patients in our medical center are of Chinese ethnicity, followed by Malay and Indian. Recently, studies have focussed on the Fc receptors in different ethnic groups and their relation to SLE. We chose to study the gene distribution of this receptor in the Chinese and Malays population in Malaysia. We designed a polymerase chain reaction-allele specific primers (PCR-ASP) method to distinguish the two allelic forms. Genomic DNA was isolated from the peripheral blood of 183 Chinese and 55 Malays SLE patients as well as 100 Chinese and 50 Malays healthy controls. Genotyping of Chinese SLE patients revealed that the gene frequencies for Fc[gamma]RIIIB-NA1 and Fc[gamma]RIIIB-NA2 were 0.648 and 0.347, while in the ethnically matched healthy controls they were 0.68 and 0.32, respectively. One out of the 183 Chinese SLE patients was identified as a NA-null due to the absence of PCR product for both alleles. The Fc[gamma]RIIIB-NA1 and Fc[gamma]RIIIB-NA2 allele frequencies for both the Malays SLE and healthy controls were 0.62 and 0.38.
Recently evidence has been provided for a genetic control of T-cell dependent cytokine production by HLA-class II. Candidate genes in multiple sclerosis, a T-cell mediated autoimmune disease, are the disease-associated DR2, DQ6, Dw2 haplotype. Previous observations by us and others imply a HLA-DR2 dependent propensity of antigen-specific T-cell lines to produce increased amounts of TNF-α/β. Here, we tested a possible association between HLA or disease status with cytokine production employing the simple and widely used method of bulk cultures. Peripheral blood cells of 48 patients and 68 healthy individuals were analyzed. We observed no significant differences of the cytokine production in relation to disease status or any HLA polymorphism. Our data indicate that, in contrast to monoclonal T-cell cultures, bulk cultures are not suitable to detect immunogenetic control of T-cell function.


The immunopharmacological characteristics of angelan, a polysaccharide purified from Angelica gigas Nakai, were investigated in relation to the specificity to immune cells. The treatment of angelan increased the expression of IL-2, IL-4, IL-6, and IFN-γ. The expression of IL-6 and IFN-γ was rapidly augmented but that of IL-2 responded later. In the case of IL-4, angelan stimulated at early time after exposure but down-regulated thereafter. These results suggested that macrophages and natural killer cells involved in nonspecific immunity were primarily activated and helper T cells were secondarily affected by angelan. Angelan also had lympho-proliferative potential to B cells, specifically. The specificity of angelan was also elucidated in a cell fractionation experiment. The activated B cells by angelan also increased antibody production. The direct activation of B cells, macrophages, and accessory cells and the indirect activation of helper T cells coordinately increased immune functions such as in vitro and in vivo T-dependent immunization and antibody production. The experiment of host resistance to syngeneic tumors also showed that angelan potentiated the immune functions. In conclusion, angelan, a purified polysaccharide from an oriental herbal drug, showed characteristic immunostimulation, which was different from clinically used polysaccharides such as lentinan and PSK.

Immunocytokines, such as interleukin-1 (IL-1), have been shown to be involved in the activation and/or induction of a variety of transcription factors which may modulate the expression of genes possessing DNA binding sites on which these transcription factors act. The promoter DNA sequence of the [mu] opioid receptor gene contains IL-1 response elements such as NF-IL6, and, therefore, the receptor gene may be responsive to IL-1. To investigate the effect of IL-1 on the opioid receptor gene, the in vitro expression of [mu] opioid receptor mRNA in neural microvascular endothelial cells (NMVEC) was determined before and after IL-1 treatment. PCR analysis revealed that there was virtually no [mu] opioid receptor expression at basal levels and no increase after either IL-1[alpha] or IL-1[beta] treatment. However, simultaneous treatment with both IL-1[alpha] and IL-1[beta] increased [mu] opioid receptor expression. This upregulation of [mu] opioid receptor expression provides direct evidence of a relationship between opioid and cytokine actions, and suggests that opioid-dependent pathways may be modulated in the disease state.

**Industrial Crops and Products (1)**


http://www.sciencedirect.com/science/article/B6T77-3V8CHK5-4/2/f4dca4097b73657b0251a4473dc7e349

A prerequisite for molecular genetic studies is the ability to isolate DNA. In species of the genus Lesquerella, high polysaccharide content makes this basic requirement difficult to achieve. The carbohydrates and the nucleic acids coprecipitate during sample preparation and form large pellets where the DNA is trapped in gum and is no longer retrievable. A DNA isolation method was developed to solve this problem. After breaking down the cell walls, the cell nuclei are separated from the cytoplasmatic and intracellular fluids by centrifugation in a viscous medium. This preparatory step separates the nuclei that contain the desired DNA from other cellular compartments containing the problem-causing carbohydrates. The DNA is then isolated from the nuclei without interference. High quality DNA was obtained and used successfully for restriction endonuclease digestion and polymerase chain reaction amplifications.

**Infection, Genetics and Evolution** (2)


http://www.sciencedirect.com/science/article/B6W8B-4CXMP6N-1/2/f59e67a951bece2a8f4a599a8a660eca

Mucosal leishmaniasis, which is a sporadic disease in the Sudan, was shown by isoenzyme
characterization and PCR to be caused by Leishmania donovani. However, it was not clear if the parasite was exactly the same strain as that causing visceral leishmaniasis (VL), or of a different strain. We utilized a new generation of molecular DNA markers, minisatellites and kinetoplast DNA, for rapid characterization of the parasite. The results show that the genotypes of some of the parasites causing VL are different from those causing mucosal leishmaniasis. The L. donovani isolates causing visceral disease, as well as post-kala-azar mucosal leishmaniasis (PKML), have been shown to possess characteristic haplotypes. However, sequencing of a portion of the cytochrome oxidase II (COII) gene indicates that the parasite that invades the oral mucosa is divergent from other parasites causing VL. It appears to possess features of a more ancestral parasite with pronounced sequence homology to L. major. This agrees with earlier studies where isolates of mucosal leishmaniasis have been shown to possess an isoenzyme profile distinct from L. donovani and a different geographical distribution, albeit often overlapping with that of L. donovani.


http://www.sciencedirect.com/science/article/B6W8B-45C1JW2-2/2/6e11ca8e750cbf012143d41ba48986ef

Shiga toxin producing Escherichia coli O103:H2/H- belong to the third most frequently isolated EHEC serotypes in Germany following isolates of O157:H7/H- and O26:H11/H-. A total of 145 respective E. coli 103 isolates from single cases of diarrhoea and haemolytic uremic syndrome (HUS) in 1997-2000 were characterised by a range of molecular subtyping methods (PFGE, P-gene profiling, ribotyping, electrotyping) and phage typing in order to analyse their genetic relatedness and the practicability for new epidemiological tracing back. All isolates cluster into a distinct EHEC subgroup and reveal a high clonal diversity together with a considerable stability. Since strains of this serotype rank up to the third most frequently isolated EHEC in Germany a large population of this serotype, and therefore, a great supply of such strains may exist in this country.

Insect Biochemistry and Molecular Biology (34)


http://www.sciencedirect.com/science/article/B6T79-3WJFBBW-4/2/3c4c5a49378e976e150875261c2baaf12

Major proteins of honey bee (Apis mellifera) royal jelly are members of the MRJP protein family. One MRJP protein termed MRJP3 exhibits a size polymorphism as detected by SDS-PAGE. In this report we show that polymorphism of the MRJP3 protein is a consequence of the polymorphism of a region with a variable number of tandem repeats (VNTR) located at the C-terminal part of the MRJP3 coding region. We present the characterization of five polymorphic alleles of MRJP3 by DNA sequencing. By PCR analyses, at least 10 alleles of distinct sizes were found in randomly sampled bees. Studies with nurse bees from a single honeybee colony revealed both Mendelian inheritance and very high variability of the MRJP3 genomic locus. The
high variability and simple detection of the MRJP3 polymorphism may be useful for genotyping of individuals in studies of the honeybee.


http://www.sciencedirect.com/science/article/B6T79-4F4WXNM-1/2/fff14ec0d6df281e2b2e8192a4edc044

Long-chain neurotoxins derived from the venom of the Buthidae scorpions, which affect voltage-gated sodium channels (VGSCs) can be subdivided according to their toxicity to insects into insect-selective excitatory and depressant toxins ([beta]-toxins) and the [alpha]-like toxins which affect both mammals and insects. In the present study by the aid of reverse-phase HPLC column chromatography, RT-PCR, cloning and various toxicity assays, a new insect selective toxin designated as Bj[alpha]IT was isolated from the venom of the Judean Black Scorpion (Buthotus judaicus), and its full primary sequence was determined:

MNYLVVICFALLLMTVVESGRDAYIADNLNCAYTCGSNSYCNTECKNGAVSGYCQWLGYGN
ACWCINLPDKVPIRIPGACR (leader sequence is underlined). Despite its lack of toxicity to mammals and potent toxicity to insects, Bj[alpha]IT reveals an amino acid sequence and an inferred spatial arrangement that is characteristic of the well-known scorpion [alpha]-toxins highly toxic to mammals. Bj[alpha]ITs sharp distinction between insects and mammals was also revealed by its effect on sodium conductance of two cloned neuronal VGSCs heterologously expressed in Xenopus laevis oocytes and assayed with the two-electrode voltage-clamp technique. Bj[alpha]IT completely inhibits the inactivation process of the insect para/tipE VGSC at a concentration of 100 nM, in contrast to the rat brain Nav1.2/[beta]1 which is resistant to the toxin. The above categorical distinction between mammal and insect VGSCs exhibited by Bj[alpha]IT enables its employment in the clarification of the molecular basis of the animal group specificity of scorpion venom derived neurotoxic polypeptides and voltage-gated sodium channels.


http://www.sciencedirect.com/science/article/B6T79-4BVNV0B-2/2/4d549717d45f7ce61d070519710e113a

Octopamine regulates multiple physiological functions in invertebrates. The biological effects of octopamine and the pharmacology of octopamine receptors have been extensively studied in the American cockroach, Periplaneta americana. This paper reports the cloning of the first octopamine receptor from Periplaneta americana. A cDNA encoding a putative 7 transmembrane receptor was isolated from the head of Periplaneta americana. The encoded protein contains 628 amino acids and has sequence similarity to other biogenic amine receptors. This protein was expressed in COS-7 cells for radioligand binding studies using the antagonist 3H-yohimbine. Competitive binding comparing biogenic amines that could potentially function as endogenous ligands demonstrated this receptor had the highest affinity for octopamine (Ki=13.3 [mu]M) followed by tyramine, dopamine, serotonin and histamine. Octopamine increased both cAMP levels (EC50=1.62 [mu]M) and intracellular concentrations of calcium through the receptor expressed in HEK-293 cells. Tyramine increased levels of both of these second messengers but only at significantly higher concentrations than octopamine. The cAMP increase by octopamine was independent of the increase in calcium. Competitive binding with antagonists revealed this receptor is similar to Lym oa1 from Lymnaea stagnalis. The data indicate that this cDNA is the first octopamine receptor cloned from Periplaneta americana and therefore has been named Pa

http://www.sciencedirect.com/science/article/B6T79-3TWYNN5-B/2/aeeed34b8dad71f5485c723d2bb6028c

The cDNA coding for a Ser-protease-related protein (Scg-SPRP) was cloned from desert locust (Schistocerca gregaria) midgut. The derived amino acid sequence consists of 260 residues and shows strong sequence similarity to insect trypsin-like molecules. It is, however, likely that Scg-SPRP is not a proteolytically active enzyme and that it plays another physiologically relevant role, since two out of three residues which are indispensable for catalytic activity of Ser-proteases are replaced. Northern analysis revealed that the Scg-SPRP gene is expressed in midgut tissue and that this expression is strongly induced in adult female locusts. Moreover, the occurrence of the transcript (1.2 kb) fluctuates during the molting cycle and during the female reproductive cycle. Juvenile hormone (JH III) dependence of transcription was investigated by chemical allatectomy (precocene I) of adult females. This resulted in inhibition of vitellogenesis and in disappearance of the Scg-SPRP transcript. Expression of Scg-SPRP in precocene-treated locusts could be reinduced by additional treatment with JH III or with 20-OH-ecdysone.


http://www.sciencedirect.com/science/article/B6T79-3W2T43D-G/2/fb33e2c28ebd12288029fee4b9a417bb

Using HPLC separation, radioimmunoassay, and subsequent bioassay, we have detected the presence of an active peptide, which co-elutes with the insect myoinhibitory peptide leucomyosuppressin, in the brain of the cockroach Diploptera punctata. We have isolated a cDNA encoding the precursor for this peptide from cDNA libraries representing D. punctata brain RNA. The cDNA sequence contains an open reading frame that upon translation would result in a prepropolypeptide of 96 amino acids. Proteolytic cleavage of the predicted precursor could result in several peptides, including a 10 amino acid C-terminal peptide that would, upon modification of the NH2 and COOH-terminal amino acids, be identical to the insect FLRFamide, leucomyosuppressin. No other RFamide products are predicted to be processed from the precursor. Southern blot analysis indicates that the gene is present in the D. punctata genome in a single copy. Northern blot analysis shows that the gene is predominantly expressed as a 3.8 kb mRNA in cockroach brain. Study of the expression of the leucomyosuppressin gene in D. punctata brain, using in situ hybridization, indicates that expression occurs primarily in the pars intercerebralis of the protocerebrum, a region showing abundant FMRFamide-like immunoreactive neurosecretory cells. Immunohistochemistry and HPLC coupled to radioimmunoassay indicates that leucomyosuppressin represents a significant proportion of FMRFamide-related peptide production in the brain. However, HPLC analysis also indicates the presence of significant levels of other related peptides, demonstrating the presence of more than one FMRFamide-related gene in this insect.
Molecular sexing methods have been devised for sexing Mediterranean fruit fly (medfly) individuals using minimal amounts of material from any stage of the life cycle. Molecular sexing methods are particularly valuable when material is obtained from pre-adult stages and sex identification based on morphological characters is not possible. These methods may also be useful for adult stage material in situations where only limited amounts or poorly preserved specimens are available. The sexing methods described here use the polymerase chain reaction (PCR) to amplify sequences known to originate from the sex chromosomes of this species. One method co-amplifies homologous regions of the ITS1 ribosomal DNA from both the X and Y chromosomes. Males and females are distinguished based on the restriction fragment pattern produced after digestion of the PCR products with the restriction enzyme ApoI. A second method identifies males based on the positive amplification of a repetitive DNA sequence originating from the Y chromosome. Both methods are shown to be capable of establishing the sex identity of individuals using only minimal amounts of material from any stage of the life cycle.

[alpha]-Amylases are important digestive enzymes in weevils that infest starchy seeds, and plants have evolved proteinaceous [alpha]-amylase inhibitors ([alpha]AIs) for protection. To gain a better understanding of the interaction between weevil [alpha]-amylases and [alpha]AIs, we cloned the [alpha]-amylase cDNA of Zabrotes subfasciatus larvae. Larvae of this bruchid infest seeds of cultivated varieties of the common bean, Phaseolus vulgaris, although the seeds contain high levels of an [alpha]AI. The [alpha]-amylase cDNA, called ZsAmy, encodes a mature protein of 466 amino acids with a signal peptide of 17 amino acids. This protein has 50-60% amino acid identity with the other five known insect [alpha]-amylases. Three amino acid residues known to be important for catalysis and three histidine residues involved in substrate binding are conserved in the derived amino acid sequence of ZsAmy. Expression of ZsAmy with a baculovirus vector in cultured insect cells resulted in the production of active [alpha]-amylase. [alpha]AI-1, the form of the inhibitor found in cultivated beans, does not inhibit larval or expressed bruchid [alpha]-amylase, but [alpha]AI-2, a form of the inhibitor found in certain wild bean accessions, does inhibit the larval, as well as the expressed bruchid [alpha]-amylase. These and other observations lead to the conclusion that ZsAmy encodes the major larval amylase of this bruchid species.
Anopheles gambiae. All five show sequence similarity to genes thought to be involved in vertebrate or invertebrate defense responses. Sp14A, Sp14D2 and Sp22D demonstrate changes in transcript abundance in response to bacteria injections. Sp14A and Sp14D2, as well as the previously characterized Sp14D1, are induced by infection with the malaria parasite, Plasmodium berghei. These three proteases, along with Sp18D, are related to a group of secreted proteases that have amino-terminal clip domains and trypsin-like substrate specificity. BLAST results and phylogenetic analyses group Sp14A, Sp14D1 and Sp14D2 with the Drosophila protease EASTER, and three prophenoloxidase activating enzymes from other insects. EASTER's substrate is SPAETZLE, a ligand involved in embryogenesis but also in activating anti-microbial peptide synthesis. Their similarity to EASTER and immune inducibility suggest that one of these proteases may activate a SPAETZLE-like ligand during anti-parasite responses in mosquitoes. Alternatively, as potential prophenoloxidase activators, Sp14A, Sp14D1 or Sp14D2 may play a role in melanotic encapsulation of Plasmodium.


http://www.sciencedirect.com/science/article/B6T79-49KS3H1-1/2/ff24497ebb6e9c120450d693250ec9e2

The distributions of mRNAs for two cuticular proteins of Hyalophora cecropia were examined with RT-PCR and in situ hybridization. For major regions of larval and pupal cuticle, there was a strong correspondence between the type of cuticle and the predominant cuticular protein message found. Epidermal cells underlying soft cuticle had mRNA for HCCP12, with a RR-1 consensus attributed to soft cuticle, while the epidermal cells associated with hard cuticle had predominantly mRNA for HCCP66, a protein with the RR-2 consensus attributed to hard cuticle. Both messages were found in all areas of the pupal fore- and hind-wings, with modest area-specific difference in concentration being much less than differences in the relative abundance of these cuticular proteins. mRNA for HCCP12 was present in imaginal discs of feeding larvae of H cecropia. Data from Bombyx mori available at SilkBase (http://www.ab.a.u-tokyo.ac.jp/silkbase/) revealed that imaginal discs from feeding larvae had abundant mRNA for RR-1 cuticular proteins, representing six distinct gene products. Only discs from spinning larvae had mRNAs that coded for RR-2 proteins arising from 10 distinct genes. Thus, lepidopteran wing imaginal discs can no longer be regarded as inactive in larval cuticle production.


http://www.sciencedirect.com/science/article/B6T79-416BXBB-F/2/f9576e4587290da188db31a1f3a0984a

Reverse transcriptase-polymerase chain reaction (PCR) was used to clone two esterase cDNAs from a diazinon-resistant field population of horn flies that expresses qualitative and quantitative differences in esterases compared with a susceptible population. The open reading frame from one of the esterase cDNAs, Hi[alpha]E7, exhibits substantial amino-acid identity to an esterase associated with diazinon resistance in Lucilia cuprina. RNA Northern blots showed that Hi[alpha]E7 mRNA was more abundant in the diazinon-resistant population than the susceptible population. DNA copy number analysis did not reveal major differences in Hi[alpha]E7 gene copy number between the two populations. The full-length cDNA to Hi[alpha]E7 was cloned and sequenced, and found to contain all of the highly conserved sequence elements associated with carboxyl/cholinesterases. The Hi[alpha]E7 homologs in diazinon-resistant strains of L. cuprina
and Musca domestica have been shown to possess an amino-acid substitution conferring diazinon hydrolytic activity to the esterase enzyme. This amino-acid substitution was not found in diazinon-resistant horn flies examined by allele-specific PCR. Individual flies from the resistant field population were phenotyped as diazinon-resistant or diazinon-susceptible by topical diazinon application bioassays and total RNA isolated and hybridized to Hi[alpha]E7 probe in ribonuclease protection assays. Hi[alpha]E7 transcript was expressed at a five-fold higher level in resistant female individual flies than in susceptible female individuals.


http://www.sciencedirect.com/science/article/B6T79-47PR7BM-2C/2/6dc92f0ab396977f3fb316c9b2c9354a

The actin protein is a critical protein in eukaryotic cells. Four actin genes, constituting what appear to be a set of muscle specific actin genes, have been isolated from the genome of the oriental fruit fly Bactrocera dorsalis. DNA sequences have been determined for the coding as well as 3' and 5' flanking regions for each of these genes. These genes have also been characterized in terms of RNA expression patterns, and comparisons have been made to actin genes from other species. Consistent with other actins, there is a high degree of amino acid sequence conservation in the coding regions of these genes. However, even within the coding regions codon usage patterns in the oriental fruit fly are quite different from some other well characterized species. In addition, the DNA sequences in the intermediate 3' and 5' flanking regions exhibit virtually no detectable sequence homology both within and between species. In terms of nitrons, three of the four actin genes from the oriental fruit fly described here have a single intervening sequence. Two of these genes share the same intron position with the two muscle specific actin genes act79B and act88F from Drosophila melanogaster and with one muscle specific actin gene CcA1 from the Mediterranean fruit fly, Ceratitis capitata. Another gene from the oriental fruit fly shares the same intron position as the muscle specific actin gene act57B from D. melanogaster. Such conservation of intron positioning between species is highly unusual among previously characterized actin genes. Using unique sequences found in the 3' untranslated regions, gene specific probes have also been constructed. These have been used to detect the expression patterns of individual genes in a temporal and spatial manner. Each of the four genes examined here show differential patterns of expression. The patterns indicate that all four genes are most likely to encode muscle specific actins.


http://www.sciencedirect.com/science/article/B6T79-3VNPHG5-1/2/1846ac7523f763944fb85efcfc48c36d

A putative crayfish iron-responsive element (IRE) is present in the 5'-untranslated region of the crayfish ferritin mRNA. The putative crayfish IRE is in a cap-proximal position and shares most of the structural features of the consensus IRE, but the RNA stem-loop structure contains a bulge of a guanine instead of a cytosine at the expected position, so far thought to be a hallmark of IREs. By using an electromobility shift assay this IRE was shown to specifically bind purified recombinant human iron regulatory protein 1 (IRP1) as well as a factor(s) present in a homogenate of crayfish hepatopancreas, likely to be a crayfish IRP1 homologue. With mutations in the crayfish IRE, the affinity of IRP to IRE was drastically decreased. A cDNA2 encoding an IRP1-like protein was cloned from the hepatopancreas of crayfish. This protein has sequence
similarities to IRP1, and contains all the active-site residues of aconitase, two putative RNA-binding regions and a putative contact site between RNA and IRP. These results show that a crayfish IRE, lacking the bulged C, can bind IRP1 in vitro and that an IRP1-like protein present in crayfish hepatopancreas may have both aconitase and RNA-binding activities.


We have cloned three cDNAs from the sweet potato hornworm Agrius convolvuli that encode precursor molecules for peptides structurally related to bombyxin, an insulin-related brain secretory peptide in Bombyx mori. The Agrius bombyxin-related peptide (ABRP) cDNAs are classified into type A and B according to their sequence similarity. The prepro-ABRPs deduced from the cDNA sequences have the insulin-like domain organization of signal peptide/B chain/C peptide/A chain. The ABRP transcripts in Agrius brain were shown to locate in four pairs of medial neurosecretory cells, the homologous group of neurosecretory cells that produce bombyxins in Bombyx brain. Genomic Southern analysis indicated the presence of multiple copies of ABRP gene in the Agrius genome. Results showed that the ABRP genes are remarkably different from the vertebrate insulin genes in the number of copy and spatial localization of the transcripts.


The kdr and super-kdr point mutations found in the insect sodium channel gene are postulated to confer knockdown resistance (kdr) to pyrethroids. Using an allele-specific PCR assay to detect these mutations in individual horn flies, Haematobia irritans (L.), we determined the allelic frequency of the kdr and super-kdr mutations in several wild and laboratory populations. Wild populations with very similar allelic frequencies had resistance levels that ranged widely from 3- to 18-fold relative to a susceptible population. Conversely, the kdr allele frequency in a lab population with 17-fold resistance was nearly double that found in a heavily pressured wild population with 18-fold resistance. We conclude that, although the kdr mutation confers significant levels of pyrethroid resistance, a substantial component of resistance in insecticidally pressured populations is conferred by mechanisms that are PBO-suppressible. High super-kdr allele frequencies were detected in two resistant lab populations, but in wild populations with equivalent resistance the super-kdr allele frequency was very low. Interestingly, in over 1200 individuals assayed, the super-kdr mutation was never detected in the absence of the kdr mutation.

Three degenerate primers were designed to match the most conserved regions within the DNA-binding domains of several selected members of the steroid hormone receptor family. Use of these primers in the polymerase chain reaction with cDNA from Galleria mellonella prepupae detected a 177 bp fragment that had 87% identity to the Manduca sexta gene MHR3 and 75% to the Drosophila melanogaster DHR3 gene, and therefore was named "GHR3". Screening of a Galleria penultimate instar cDNA library with this fragment yielded a cDNA clone that contained a 557 condon open reading frame, predicting a 62.3 kDa protein. The deduced amino acid sequence of GHR3 showed 92% overall identity with the MHR3 protein and 97 and 70% identity with DHR3 in the putative DNA- and ligand-binding domains, respectively. Hybridization of whole body RNA revealed high GHR3 mRNA levels during both the larval and pupal molts, coincident with the molt-inducing ecdysteroid pulses, and low or undetectable levels during the first half of the last instar. During the larval-pupal transformation, no GHR3 mRNA was found at the beginning of the stemmatal pigment retraction at the onset of the ecdysteroid rise; maximal levels were observed 4 h later, coincident with the peak ecdysteroid titer (over 2.3 [mu]g 20E equivalents/ml hemolymph). Two mRNAs (4.6 and 3.6 kb) were detected when the ecdysteroid titer was high. Injection of 2 [mu]g/gm 20E into isolated final instar larval abdomens induced the appearance of the 4.6 kb mRNA within 1.5 h; the mRNA level then reached maximum by 3 h and declined by 6 h. No 3.6 kb mRNA was detectable during that time. A 10-fold lower 20E dose caused only trace induction by 3 h.


The immune state of insects is defined by a set of proteins that is absent in the naive state. To explore the immune system of Trichoplusia ni in more detail we have employed a PCR differential...
display technique to compare the mRNA population of untreated last instar larvae to that of immunized animals. In the primary display, more than one hundred bands seemed induced upon bacterial challenge. When they were used as probes in Northern blots, 35% of these probes detected inducible mRNA species. Such probes were used to screen a cDNA library from immunized larvae. We isolated clones for T. ni homologs of cecropin A, lysozyme and attacin. One differentially expressed band hybridized to clones for BJHSP1, a hemacyanin-related protein which is hormonally up-regulated in last instar larvae; this induction is probably not related to the bacterial infection. Still other probes recognized inducible mRNAs of 1.6 and 1.0 kb. The corresponding cDNA clones did not show strong sequence homology to any known proteins. We have demonstrated the potential of this PCR technique to display both known and unknown genes specific for the immune state of whole insects against a background of genes involved in larval development.


http://www.sciencedirect.com/science/article/B6T79-4DR1FRH-1/2/ff7b6bdf7c205d104aa5cf0d10efe65

Two defensins showing high mutual similarity have previously been characterized in honeybee Apis mellifera: royalisin, a peptide isolated from the royal jelly, and defensin, found in the hemolymph of bacterially infected bees. Here we show that both these peptides are encoded by the same polymorphic gene, which we termed defensin1. Besides this gene, we identified an additional defensin gene coding for a novel honeybee defensin designated defensin2. The pre-pro-peptide sequence of defensin 2 was inferred from its cDNA. Mature defensin 2 peptide shows 55.8% identity with defensin 1. Sequences of genomic loci of the two defensin genes revealed their different structure. Defensin1 possesses an exon-intron structure unique among arthropoda defensin genes. Its second intron splits exactly the common structural module of defensins from a short amidated C-terminal extension found only in hymenopteran defensins. Transcription of defensin genes in some nurse honeybees tissues was studied by RT-PCR. Both defensins are expressed in heads and thoraces. Defensin1 but not defensin2 mRNA was detected in hypopharyngeal, mandibular and thoracic salivary glands. Immune response elements were identified by computer analysis of the promoter regions of defensin genes. Their different representation in these genes reflects presumably observed tissue-specific expression of defensins.


http://www.sciencedirect.com/science/article/B6T79-3YF4B49-G/2/0b081bb0d59599924f90b9c35c3ed518

Three potent insecticidal peptide toxins were purified from the venom of the primitive weaving spider, Diguetia cavities. The toxins share significant homology (> 40%) in their amino acid sequences and are of related size (masses of 6371-7080 Da). In lepidopteran larvae, the toxins cause a progressive spastic paralysis, with 50% paralytic doses (PD50s) ranging from 0.38 to 3.18 nmol/g, suggesting them to be among the most potent insecticidal compounds yet described from arthropod venoms. The most potent of these toxins, DTX9.2, was cloned using a reverse transcription-polymerase chain reaction (RT-PCR). The cDNA encodes a 94 amino acid precursor which is processed to the active 56 amino acid peptide by removal of a signal and propeptide sequence. The gene encoding DTX9.2 was isolated and characterized. The
transcriptional unit spans 5.5 kilobases and is segregated into five exons. DNA sequences upstream from the first exon contain a TATA box and two palindromic sequences (one with homology to a CAAT consensus) which together may constitute a functional promoter. The highly segmented gene structure observed for this small peptide suggests that a mechanism such as exon shuffling may have played a role in the evolution of this toxin family.


http://www.sciencedirect.com/science/article/B6T79-42M1D7K-4/2/e3bdf4722bd5b805d2152a90e30fea3f

In many insects, semen coagulates into a mating plug at the distal part of the female's genital tract. Mating plugs have been proposed to facilitate sperm movement or to prevent subsequent matings or sperm loss. The molecular constituents of insect mating plugs have not previously been characterized. Here we report that an abundant autofluorescent protein made by the Drosophila melanogaster male's ejaculatory bulb is a major constituent of the posterior region of the mating plug. Identities in size, chromosomal location and expression pattern indicate that the autofluorescent protein is PEB-me, an abundant ejaculatory bulb protein reported by Ludwig et al. [Biochem. Genet. 29 (1991) 215]. We cloned and sequenced the RNA encoding this protein. The transcript, which is male-specific and expressed only in the ejaculatory bulb, encodes a 377 a.a. predicted secreted protein with PGG repeats similar to those in homopolymer-forming proteins found in spider silk.


http://www.sciencedirect.com/science/article/B6T79-3W49339-7/2/d7b46be065a5c84815fdd86690ecc2bbc

A 185-kDa silk protein (sp185) from Chironomus tentans, present in both larval and prepupal silks, contains a striking amino acid sequence motif, Cys-X-Cys-X-Cys, which occurs about every 22-26 residues. Homologous proteins have been found in Chironomus pallidivittatus (sp185) and Chironomus thummi (sp220), which apparently differ in size but are very similar in overall composition and sequence. While surveying Australasian species of Chironomus and Kiefferulus we obtained evidence for immunologically related silk proteins having similar size and amino acid composition, but noticeably less Cys. Interspecies in situ hybridization to polytene chromosomes with C. tentans and C. pallidivittatus cDNA probes indicated that each species had a related gene. One pair of C. tentans cDNA-derived primers enabled polymerase chain reaction amplification of a discrete fragment of this gene from Kiefferulus 'cornishi'. Preliminary sequence information for this fragment confirmed the presence of an encoded Cys-X-Cys-X-Cys motif in what appeared to be a similar protein region containing less Cys. We conclude that homologs of C. tentans sp185 and its gene have been identified which may contain significant deviations in structure. Once suitable libraries are available, probes described here will be useful for selecting cDNA and genomic clones for detailed study.

The single intron of the heavy-chain fibroin gene in domesticated (Bombyx mori) and wild (B. mandarina) silk moths has a length of approximately 1000 nucleotides. It is located only 57 bp from the gene's core promoter and harbors multiple AT-rich regulatory elements that have been found to enhance the basal level of transcription in vitro. In this work, the intronic nucleotide variability among members of both Bombyx species is analyzed. The intron sequences of B. mori strains k120 and Nistari, as well as B. mandarina specimens from Japan and Korea, were obtained. This information was compared with the previously reported sequences of B. mori strains p50 and C-108, as well as an additional B. mandarina specimen collected in Japan. We found a total of 26 variant positions, including variants shared by members of both species and species-specific changes. The potential functional role of these variants was investigated by using the program MatInspector to search for putative binding sites of transcription factors within the intron. We detected a multitude of putative binding elements distributed along the entire intronic sequence. Among them, 22 correspond to protein binding domains that are known to regulate fibroin transcription. The mapping of multiple variant positions within these putative binding sequences as well as in known regulatory elements of the intron argue for functional significance on the regulation of transcription.


The wing-deficient mutant, flugellos (fl), of the silkworm lacks four wings in the pupa and the adult, due to aberrant wing morphogenesis during metamorphosis. To elucidate the mechanisms of wing-specific deficiencies in the fl mutant, we used mRNA differential display and identified five genes abnormally expressed in the fl wing discs. Northern blot and RT-PCR analyses revealed that four genes were overexpressed, but the fifth one was not transcribed in the fl wing discs. The expression level of ribosome-associated protein p40 in the fl wing discs was elevated approximately 10 times compared to the wild-type (WT) discs. Another overexpressed gene CB10 encodes a novel wing-specific protein with a putative zinc-finger motif. Overexpression of two components of extracellular matrix, cuticle protein 18 (BMCP18) and a fibrillin-like protein AD10, may result in the abnormal wing morphogenesis in the fl mutant. In contrast, a novel member of multifunctional Ca2+-binding protein annexins, designated as annexin b13 (Anx b13), was expressed dominantly in the wing discs of WT but completely repressed in the fl tissues. Strong expression of Anx b13 in wing discs during the fourth and fifth instar indicates that ANX B13 plays an important role in wing morphogenesis.

hybridization to the polytene chromosomes. Of the six introns present, four are positioned identically to those of the Drosophila homolog, one is similarly positioned, and one is novel. A 1955 nt cDNA potentially encodes a 392 amino acid protein of an estimated 45 kDa. Amino acid comparisons between the deduced protein and previously known tryptophan oxygenases revealed 74% identity between Anopheles and Drosophila, and 53% identity between Anopheles and nematode or mammalian proteins. Northern analysis detected a developmentally regulated transcript about 2 kb in length. Since this gene is known to control adult eye color in other flies, its cloning from A. gambiae provides the basis for a dominant phenotypic marker for germline transformation, one whose expression, unlike that of white, is not cell autonomous.


http://www.sciencedirect.com/science/article/B6T79-47PR7VD-6M/2/8fc0591c2fdea95bde8a942c21ccf96e

A cDNA clone containing a 921 bp open-reading frame (307 amino acids; 34 kDa) homologous to the TATA-binding protein (TBP) was isolated and sequenced from a Spodoptera frugiperda cell line that is commonly used in the baculovirus expression system. Analysis of the S. frugiperda TBP (SfTBP) sequence showed that the amino-terminal portion of SfTBP diverged significantly from that of other TBP sequences including Drosophila melanogaster whereas the carboxy-terminal sequence was highly conserved. Southern blot analysis indicated that SfTBP was encoded by a single gene in the S. frugiperda genome. Northern blot analysis indicated that steady-state levels of the 1.3 kb SfTBP transcript declined by 24 h post-infection corresponding to the time of virus-induced inhibition of host-cell transcription. Corresponding western blot analysis showed that TBP protein levels remain constant up to 72 h post-infection.


http://www.sciencedirect.com/science/article/B6T79-3T6YH4M-4/2/8124b8bd4a212f7b8a04fe66995098ff

The gut of most insects is lined with a semi-permeable peritrophic membrane (or peritrophic matrix) composed of chitin, proteoglycans and proteins. Despite the probable importance of the peritrophic membrane in facilitating the digestive process and protecting insects from invasion by micro-organisms and parasites, there has been little characterization of the specific components and their interactions within this acellular structure. Here we report the characterization of an integral peritrophic membrane glycoprotein, peritrophin-48, from the larvae of the fly Lucilia cuprina, a primary agent of cutaneous myiasis in sheep. Peritrophin-48 was purified from peritrophic membrane obtained by larval culture and its location within the peritrophic membrane determined by immuno-fluorescence and immuno-gold localizations. The cDNA coding for peritrophin-48 was cloned and sequenced. The deduced amino acid sequence codes for a protein of 375 amino acids containing an amino-terminal signal sequence followed by five similar, but non-identical domains, each approximately 65-70 amino acids in length and characterised by a specific register of six cysteines. The deduced amino acid sequence shows significant similarity to two other peritrophic membrane proteins, peritrophin-95 and peritrophin-44, from the same species. A reverse transcriptase-PCR approach indicated that there are several highly related peritrophin-48 genes expressed in each individual. Reverse transcriptase-PCR also demonstrated the expression of peritrophin-48 in all three larval instars and adults but not pupae
or eggs. Peritrophin-48 was expressed only by the cardia and by the larval midgut. A simple structural model of a basic unit of a type 2 peritrophic membrane is presented.


http://www.sciencedirect.com/science/article/B6T79-3VGRRV2-K/2/e83efc2d7a503583930c0379e07bb3f0

Three major red hemoproteins, named RpSG I, II (identical with prolixin-S) and III, in the salivary glands of the blood-sucking insect, Rhodnius prolixus, show homology in N-terminal amino acid (AA) sequences, and are immunologically related. We focussed on one of these proteins, RpSG-I, in this paper. RpSG-I in fresh salivary gland extract was separated into two components (Ia and Ib) by isoelectric focussing gel electrophoresis. Absorption spectra of RpSG-Ia and Ib showed Soret peaks at 400 nm and 420 nm, respectively, suggesting that they are nitric oxide (NO)-unbound and -bound hemoproteins and function as NO-carriers. RpSG-I is stage-specific in appearance, being absent in 3rd and 4th instar nymphs, appearing and increasing gradually in 5th (last) instar nymphs after engorgement, and present in the adult stage. We purified RpSG-I from salivary gland extract by size exclusion and ion exchange HPLCs. It is a single electrophoretic band with an absorption peak at 400 nm, representing the NO-unbound molecule. Full-size cDNA of RpSG-I was cloned by screening with a specific polyclonal antibody from a salivary gland cDNA library. Sequence analysis of RpSG-I cDNA showed an open reading frame encoding a signal peptide (23 AA) and mature protein (179 AA) of 19778 daltons. The deduced N-terminal AA sequence of the RpSG-I was identical with that of the hemoprotein reported as nitrophorin-3 (Champagne et al., 1995).


http://www.sciencedirect.com/science/article/B6T79-44B29F1-3/2/e2f9c0414d8f66b85b1f7cc1ce574458

When insects molt, the exoskeleton is renewed under the controls of insect hormones via the biosynthesis and degradation of cuticle proteins. To understand the hormonal control of cuticle formation, we used the differential display method to look for stage-specific cuticle genes, and identified a novel cDNA named Bombyx mori Cuticle Protein GlyGlyTyr-repeat 1 (BMCPG1). Expression of BMCPG1 mRNA peaked sharply immediately after a pulse of ecdysteroid during the fourth molt and pre-pupal stages, concurrent with the expression of genes for FTZF1 and dopa decarboxylase. BMCPG1 was expressed only in the epidermis, but not in any other tissue. We cultured the larval epidermis and found that BMCPG1 expression is not induced by the continuous presence of ecdysteroid. Removal of ecdysteroid from the medium, which constitutes a pulse treatment, is required for the induction of BMCPG1 transcription. These results explain well the stage-specific expression of BMCPG1 by ecdysteroid in vivo. Based on its expression patterns and unique structure, we propose that BMCPG1 may be a novel component of epicuticle of B. mori, and is probably involved in cross-linking of proteins via its GGY repeats.

Tellam, R. L., C. Eisemann, et al. (2000). "The intrinsic peritrophic matrix protein peritrophin-95 from larvae of Lucilia cuprina is synthesised in the cardia and regurgitated or excreted as a highly
The intrinsic peritrophic matrix glycoprotein, peritrophin-95, from the midgut of larvae of Lucilia cuprina can only be solubilized from the matrix using strong denaturants. This suggests that the protein has a structural role in the matrix. Consistent with this is the finding that immuno-gold and immuno-fluorescence localizations of the protein showed a uniform distribution within the peritrophic matrix. RT-PCR demonstrated that expression of peritrophin-95 mRNA was restricted to the larval cardia, a small organ located in the anterior midgut from which the type 2 peritrophic matrix originates. Immuno-blots and ELISAs demonstrated that the sera from sheep infested naturally or artificially with these larvae recognised peritrophin-95. This indicates that peritrophin-95 stimulates the ovine immune system during larval infestation even though the protein is firmly attached to the peritrophic matrix in the larval midgut and seemingly "concealed" from the ovine immune surveillance system. Analyses of larval regurgitated or excreted material by immuno-blots, immuno-affinity purification and amino-terminal sequencing demonstrated the presence of soluble monomeric peritrophin-95. These results indicate that peritrophin-95, a candidate vaccine antigen for use in sheep is not a "concealed" antigen as previously thought. The presence of soluble peritrophin-95 in the regurgitated/excreted material from larvae suggests that this protein may be involved in a maturation phase of peritrophic matrix production, a by-product of which is the excretion or regurgitation of soluble peritrophin-95.


The peritrophic matrix lines the midgut of most insects and has important roles in digestion, protection of the midgut from mechanical damage and invasion by micro-organisms. Although a few intrinsic peritrophic matrix proteins have been characterised, no direct homologues of any of these proteins have been found in other insect species, even closely related species, suggesting that the peritrophic matrix proteins show considerable sequence divergence. We now report the identification of the cDNA and genomic DNA sequences of a Chrysomya bezziana homologue of the Lucilia cuprina intrinsic peritrophic matrix protein, peritrophin-48. The gene for C. bezziana peritrophin-48 spans 1315 bp and consists of three exons (65, 560 and 690 bp, respectively) separated by introns of 566 and 72 bp. The transcriptional start site, identified by a consensus of cDNA clones and primer extension analysis, is probably located 58 bp upstream from the start codon. However, there may be multiple start sites for transcription. Two potential TATA boxes and a consensus arthropod transcription initiator are located within 134 bp of sequence upstream of the putative transcriptional start site suggesting that this region contains the gene promoter. Immuno-fluorescence localization demonstrated that C. bezziana peritrophin-48 was localised to the larval peritrophic matrix. Protein fold recognition analysis indicated structural similarities between peritrophin-48 and wheatgerm lectin. As wheatgerm lectin binds chitin, this result suggested that C. bezziana peritrophin-48 may also bind chitin, a constituent of the peritrophic matrix. Chitin binding studies with a recombinant peritrophin-48 protein confirmed that it binds chitin. A Drosophila melanogaster homologue of peritrophin-48 encoded in an EST and a genomic sequence was also identified. The pairwise percentage identities of the deduced amino acid sequences for the peritrophin-48 homologues from the three higher Dipteran species were relatively low, ranging between 32 and 42%. Despite this sequence variability, the predicted structure of these proteins, dictated by five domains, each containing a characteristic distribution of six cysteines, was strictly conserved. It is concluded that considerable sequence variation can
be tolerated in this protein because of the constraints imposed on the structure of the protein by an extensive disulphide bonded framework.


http://www.sciencedirect.com/science/article/B6T79-4967CHH-1/2/6b1c6d10c779a20de62c765d3cda7f1a

The post-integration behavior of insect gene vectors will determine the types of applications for which they can be used. Transposon mutagenesis, enhancer trapping, and the use of transposable elements as genetic drive systems in insects requires transposable elements with high rates of remobilization in the presence of transposase. We investigated the post-integration behavior of the Mos1 mariner element in transgenic Aedes aegypti by examining both germ-line and somatic transpositions of a non-autonomous element in the presence of Mos1 transposase. Somatic transpositions were occasionally detected while germ-line transposition was only rarely observed. Only a single germ-line transposition event was recovered after screening 14,000 progeny. The observed patterns of transposition suggest that Mos1 movement takes place between the S phase and anaphase. The data reported here indicate that Mos1 will be a useful vector in Ae. aegypti for applications requiring a very high degree of vector stability but will have limited use in the construction of genetic drive, enhancer trap, or transposon tagging systems in this species.


http://www.sciencedirect.com/science/article/B6T79-42M1D7K-5/2/53b67de498c43f389992b54ae3d46389

Muscle fatty acid binding protein (FABP) is a major cytosolic protein in flight muscle of the desert locust, Schistocerca gregaria. FABP expression varies greatly during development and periods of increased fatty acid utilization, but the molecular mechanisms that regulate its expression are not known. In this study, the gene coding for locust muscle FABP was amplified by PCR and cloned, together with 1.2 kb of upstream sequence. The sequence coding for the 607 bp cDNA is interrupted by two introns of 12.7 and 2.9 kb, inserted in analogous positions as the first and third intron of the mammalian homologues. Both introns contain repetitive sequences also found in other locust genes, and the second intron contains a GT-microsatellite. The promoter sequence includes a canonical TATA box 24 bp upstream of the transcription start site. The upstream sequence contains various potential myocyte enhancer sequences and a 160 bp segment that is repeated three times. In database searches in the genome database of Drosophila melanogaster, a gene with the same gene organization and promoter structure was identified, likely the dipteran homologue of muscle FABP. Upstream of both insect genes, a conserved 19 bp inverted repeat sequence was detected. A similar but reverse palindrome is also present upstream of all mammalian heart FABP genes, possibly representing a novel element involved in muscle FABP expression.

FABP is the most abundant cytosolic protein in developed flight muscle of adult locusts, but it is completely absent in nymphs. During the two weeks following adult ecdysis, FABP rises to 18% of the total soluble proteins. Its mRNA increases steadily up to day 8, before it gradually declines until reaching a low concentration at day 15, which remains constant for the rest of the animal's life. Using a PCR primer combination specific for a 597 bp sequence of intron 1, we have developed a reverse transcription PCR assay to quantify the amount of primary transcript present in muscle tissue at various ages. The FABP gene is not transcribed prior to metamorphosis; its primary transcript rises rapidly during the first two days of adult life, and remains close to maximal until day 5. Subsequently its level rapidly declines, ultimately reaching values of less than 0.02% of the maximal level. The correlation between primary transcript, mRNA and FABP content were analyzed by modeling transcription, translation and degradation with computer modeling software. The computer simulation is in good agreement with the experimentally obtained data, suggesting that the control of FABP expression in locust flight muscle occurs predominantly at the level of transcription initiation.


A cDNA encoding acetylcholinesterase (AChE, EC 1.1.1.7) was cloned from a cDNA library constructed from an insecticide-susceptible strain of Colorado potato beetle, Leptinotarsa decemlineata (Say). The complete amino acid sequence of AChE deduced from the cDNA consisted of 29 residues for the putative signal peptide and 600 residues for the mature protein with a predicted molecular weight of 67,994. Northern blot analysis of poly(A) RNA showed an approx 13.1-kb transcript. The mature protein sequence had 57 and 61% of amino acid residues identical to those of Drosophila melanogaster and Anopheles stephensi, respectively, and produced a remarkably similar hydropathy profile when compared to those of the two dipterous species. The three residues (Ser, Glu and His) that putatively form the catalytic triad and the six Cys that form intra-subunit disulfide bonds were completely conserved when compared to the other seven AChEs from a broad range of animal species reported to date. Other properties of the deduced protein of AChE, including molecular weight and amino acid composition, agreed well with those of a previously reported study on the purified AChE from the same insect species. All these features firmly established that the cloned cDNA encodes AChE in Colorado potato beetle.


http://ijs.sgmjournals.org/cgi/content/abstract/52/3/757


http://ijs.sgmjournals.org/cgi/content/abstract/52/6/2241


http://ijs.sgmjournals.org/cgi/content/abstract/52/1/195


http://ijs.sgmjournals.org/cgi/content/abstract/52/6/2261


http://ijs.sgmjournals.org/cgi/content/abstract/54/2/583

Sixty strains of Gram-negative, anaerobic, rod-shaped bacteria from human sources initially assigned to Leptotrichia buccalis (n=58) and Leptotrichia pseudobuccalis' (n=2) have been subjected to polyphasic taxonomy. Full-length 16S rDNA sequencing, DNA-DNA hybridization, RAPD, SDS-PAGE of whole-cell proteins, cellular fatty acid analysis and enzymic/biochemical tests supported the establishment of four novel Leptotrichia species from this collection, Leptotrichia goodfellowii sp. nov. (type strain LB 577T=CCUG 32286T=CIP 107915T), Leptotrichia hofstadii sp. nov. (type strain LB 23T=CCUG 47504T=CIP 107917T), Leptotrichia shahii sp. nov. (type strain LB 377T=CCUG 47503T=CIP 107916T) and Leptotrichia wadei sp. nov. (type strain LB 167T=CCUG 47505T=CIP 107918T). Light and electron microscopy showed that the four novel species were Gram-negative, non-spore-forming and non-motile rods. L. goodfellowii produced arginine dihydrolase, (beta)-galactosidase, N-acetyl-(beta)-glucosaminidase, arginine arylamidase, leucine arylamidase and histidine arylamidase. L. shahii produced (alpha)-arabinosidase. L. buccalis and L. goodfellowii fermented mannose and were (beta) - galactosidase-6-phosphate positive. L. goodfellowii, L. hofstadii and L. wadei were (beta) - haemolytic. L. buccalis fermented raffinose. With L. buccalis, L. goodfellowii showed 3
dot}8-5\%\ DNA-DNA\ relatedness,\ L.\ shahii\ showed\ 24\%\ relatedness,\ L.\ hofstadii\ showed\ 27\%\ relatedness\ and\ L.\ wadei showed\ 24\%\ relatedness.\ 16S\ rDNA\ sequencing\ demonstrated\ that\ L.\ hofstadii,\ L.\ shahii,\ L.\ wadei\ and\ L.\ goodfellowii each\ formed\ individual\ clusters\ with\ 97, 96, 94 and \% similarity, respectively, to \ L.\ buccalis.


http://ijs.sgmjournals.org/cgi/content/abstract/52/6/1929


http://ijs.sgmjournals.org/cgi/content/abstract/53/3/669

To further investigate the diversity of micro-organisms capable of conserving energy to support growth from dissimilatory Fe(III) reduction, Fe(III)-reducing micro-organisms were enriched and isolated from subsurface sediments collected in Oyster Bay, VA, USA. A novel isolate, designated T118T, was recovered in a medium with lactate as the sole electron donor and Fe(III) as the sole electron acceptor. Cells of T118T were Gram-negative, motile, short rods with a single polar flagellum. Strain T118T grew between pH 6 and 7, with a temperature range of 4-30 °C. The optimal growth temperature was 25 °C. Electron donors utilized by strain T118T with Fe(III) as the sole electron acceptor included acetate, lactate, malate, propionate, pyruvate, succinate and benzoate. None of the compounds tested was fermented. Electron acceptors utilized with either acetate or lactate as the electron donor included Fe(III)-NTA (nitrilotriacetic acid), Mn(IV) oxide, nitrate, fumarate and oxygen. Phylogenetic analysis demonstrated that strain T118T is most closely related to the genus Rhodoferax. Unlike other species in this genus, strain T118T is not a phototroph and does not ferment fructose. However, phototrophic genes may be present but not expressed under the experimental conditions tested. No Rhodoferax species have been reported to grow via dissimilatory Fe(III) reduction. Based on these physiological and phylogenetic differences, strain T118T (=ATCC BAA-621T=DSM 15236T) is proposed as a novel species, Rhodoferax ferrireducens sp. nov.


http://ijs.sgmjournals.org/cgi/content/abstract/53/6/2073

Five strains of halophilic, Gram-negative marine bacteria (KMM 3809T, KMM 3814, KMM 3815, KMM 3817 and KMM 3818) were isolated from sediments collected from Chazhma Bay, Sea of Japan. Phylogenetic 16S rRNA gene sequence-based analysis placed these bacteria in a clade within the genus Marinobacter in the(gamma) -Proteobacteria. KMM 3809T showed highest 16S rRNA gene sequence similarity of 97\%\ to Marinobacter litoralis and 96\%\ to Marinobacter hydrocarbonoclasticus and Marinobacter aquaeolei. DNA-DNA hybridization between the five isolates was at the conspecific level (94-96 \%) and that among the closest...
phylogenetic neighbours ranged from 45\% to 62\% The new organisms were susceptible to polymyxin. Predominant fatty acids were C16: 0, C16: 1(omega)9c, C16: 1(omega)7c and C18: 1(omega)9c. Phylogenetic evidence, along with phenotypic and genotypic characteristics, showed that the bacteria constituted a novel species of the genus Marinobacter. The name Marinobacter excellens sp. nov. is proposed for this species, with the type strain KMM 3809T (=CIP 107686T).


The term flexispira' refers to micro-organisms with a particular morphology: fusiform-shaped with helical periplasmic fibrils and bipolar tufts of sheathed flagella. Two flexispira taxa have been formally named, Helicobacter bilis and Helicobacter trogontum, a third named species is Helicobacter aurati and eight additional 16S rRNA sequence-based flexispira taxa have been described by Dewhirst et al. (Int J Syst Evol Microbiol 50, 1781-1787, 2000) and given the provisional designation Helicobacter sp. flexispira taxa 1-5, 7, 8 and 10. In the present study, seven gastric or intestinal flexispira isolates from seven Finnish pigs originating from different farms were characterized. Morphologically, all these porcine isolates had typical flexispira morphology. Analysis of the 16S rDNA sequences of five isolates showed that they were most closely related to the sequences of flexispira taxa 4 and 5 and H. trogontum (taxon 6), but less closely related to taxa 1-3 and 8, H. bilis and H. aurati. Phenotypic characterization, analysis of RFLPs of 16S and 23S rDNAs and SDS-PAGE profiles revealed that all of the porcine isolates, reference strains of flexispira taxa 1, 4 and 5 and the type strain of H. trogontum (ATCC 700114T) had highly related characteristics that differed from those of the reference strains of taxa 2, 3 and 8 and H. bilis. Furthermore, a high DNA-DNA binding rate was found, in dot-blot hybridization studies, between the Finnish porcine strains, taxa 1, 4 and 5 reference strains and H. trogontum ATCC 700114T. In conclusion, polyphasic characterization of novel porcine flexispira isolates and previously described taxa 1, 4 and 5 reference strains showed that they all belong to a validly described species, H. trogontum, and that the taxonomy of known flexispiras is less complicated than proposed on the basis of 16S rDNA sequence analysis.


The taxonomic status of 64 strains of whorl-forming Streptomyces (formerly Streptoverticillium) species was re-evaluated and strains were reclassified on the basis of their phenotypes, DNA-DNA hybridization data and partial sequences of gyrB, the structural gene of the B subunit of DNA gyrase. These strains, which consisted of 46 species and eight subspecies with validly published names and 13 species whose names have not been validly published [including 10 strains examined by the International Streptomyces Project (ISP)], were divided into two groups, namely typical and atypical whorl-forming Streptomyces species, based on their phenotypes and gyrB gene sequences. The typical whorl-forming species (59 strains) were divided into six major clusters of three or more species, seven minor clusters of two species and five single-member clusters, based on the threshold value of 97 % gyrB sequence similarity. Major clusters were typified by Streptomyces abikoensis, Streptomyces cinnamoneus, Streptomyces distallicus,
Streptomyces griseocarneus, Streptomyces hiroshimensis and Streptomyces netropsis. Phenotypically, members of each cluster resembled each other closely except for the S. distallicus cluster, which was divided phenotypically into the S. distallicus and Streptomyces stramineus subclusters, and the S. netropsis cluster, which was divided into the S. netropsis and Streptomyces eurocidicus subclusters. Strains in each minor cluster closely resembled each other phenotypically. DNA-DNA relatedness between the representative species and others in each major cluster and/or subcluster, and between strains in the minor clusters, was >70 %, indicating that the major clusters and/or subclusters and the minor clusters each comprise a single species. It was concluded that 59 strains of typical whorl-forming Streptomyces species consisted of the following 18 species, including subjective synonym(s): S. abikoensis, Streptomyces arsus, Streptomyces blastmuceticus, S. cinnamoneus, S. eurocidicus, S. griseocarneus, S. hiroshimensis, Streptomyces lilacinus, Streptomyces luteoreticuli, Streptomyces luteosporeus, Streptomyces mashuensis, Streptomyces mobaraensis, Streptomyces morookaense, S. netropsis, Streptomyces orinoci, S. stramineus, Streptomyces thioluteus and Streptomyces viridiflavus.


http://ijs.sgmjournals.org/cgi/content/abstract/53/3/771

Among a large collection of South African dairy isolates, a novel Chryseobacterium taxon (DNA group 3) was previously delineated by a polyphasic taxonomic study (Hugo et al., Syst Appl Microbiol 22, 586-595, 1999). In the present paper, this taxon is further characterized using 16S rDNA sequencing, fatty acid methyl ester analysis and a comparative phenotypic analysis, resulting in the proposal of a novel species, Chryseobacterium joostei sp. nov. (type strain Ix 5aT=LMG 18212T=CCUG 46665T).


http://ijs.sgmjournals.org/cgi/content/abstract/54/3/705

Four light-yellow-pigmented, Gram-negative, short-rod-shaped, non-motile isolates were obtained from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The isolates studied were chemo-organotrophic, alkalitolerant and mesophilic. Polar lipids were analysed and phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids were 15:0, i15:0, a15:0, i15:1 and 15:1(n-6). The DNA G+C contents of the four strains were 34(middle dot)0-34(middle dot)4 mol%. The level of DNA relatedness of the four isolates was conspecific (88-98 %), indicating that they belong to the same species. The 16S rDNA sequence of strain KMM 3553T was determined. Phylogenetic analysis revealed that KMM 3553T formed a distinct phylectic line in the phylum Bacteroidetes, class Flavobacteria in the family Flavobacteriaceae and that, phylogenetically, this strain could be placed almost equidistant from the genera Gelidibacter and Psychroserpens (16S rRNA gene sequence similarities of 94 %). On the basis of significant differences in phenotypic and chemotaxonomic characteristics, it is suggested that the isolates represent a novel species in a new genus; the name Formosa algae gen. nov., sp. nov. is proposed. The type strain is KMM 3553T (=CIP 107684T).

Ivanova, E. P., R. Christen, et al. (2004). "Brevibacterium celere sp. nov., isolated from degraded thallus
Two whitish yellow, Gram-positive, non-motile, aerobic bacteria were isolated from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The bacteria studied were chemo-organotrophic, mesophilic and grew well on nutrient media containing up to 15 % (w/v) NaCl. The DNA G+C content was 61 mol%. The two isolates exhibited a conspecific DNA-DNA relatedness value of 98 %, indicating that they belong to the same species. A comparative analysis of 16S rRNA gene sequences revealed that strain KMM 3637T formed a distinct phylectic lineage in the genus Brevibacterium (family Brevibacteriaceae, class Actinobacteria) and showed the highest sequence similarity (about 97 %) to Brevibacterium casei. DNA-DNA hybridization experiments demonstrated 45 % binding with the DNA of B. casei DSM 20657T. Physiological and chemotaxonomic characteristics (meso-diaminopimelic acid in the peptidoglycan, major cellular fatty acids 15: 0ai and 17: 0ai) of the bacteria studied were consistent with the genomic and phylogenetic data. On the basis of the results of this study, a novel species, Brevibacterium celere sp. nov., is proposed. The type strain is KMM 3637T (=DSM 15453T=ATCC BAA-809T).


Two marine bacterial strains, KMM 3823T and KMM 3836, isolated from a sipuncula (Phascolosoma japonicum), a common inhabitant of Troitsa Bay in the Gulf of Peter the Great (Sea of Japan), were studied. Comparative 16S rRNA gene sequence-based phylogenetic analysis placed these bacteria into a separate branch of the Gammaproteobacteria within members of the genus Shewanella. KMM 3823T showed the highest similarity (96%[middle dot]6 %) with Shewanella fidelis. The DNA G+C contents of the two strains studied were 43[middle dot]0 mol%. The level of DNA homology between these two strains was conspecific (93 %), indicating that they represent a single genospecies. These organisms were greenish-brown, Gram-negative, polarly flagellate, facultatively anaerobic, mesophilic (temperature range 4-30 {degrees}C), neutrophilic, haemolytic and were able to degrade elastin, gelatin and DNA. They were susceptible to ampicillin, carbenicillin, gentamicin and kanamycin. The predominant fatty acids were characteristic for shewanellas: 13: 0-i, 15: 0-i and 16: 1(n-7); up to 6[middle dot]7 % of eicosapentaenoic fatty acid, 20: 5(n-3), was produced during growth at 28 {degrees}C. Phylogenetic evidence, confirmed by DNA hybridization and phenotypic characteristics revealed that the two bacteria studied constitute a new species, Shewanella waksmanii sp. nov., the type strain of which is KMM 3823T (=CIP 107701T=ATCC BAA-643T).


Aster yellows (AY) group (16SrI) phytoplasmas are associated with over 100 economically important diseases worldwide and represent the most diverse and widespread phytoplasma group. Strains that belong to the AY group form a phylogenetically discrete subclade within the phytoplasma clade and are related most closely to the stolbur phytoplasma subclade, based on analysis of 16S rRNA gene sequences. AY subclade strains are related more closely to their culturable relatives, Acholeplasma spp., than any other phytoplasmas known. Within the AY subclade, six distinct phylogenetic lineages were revealed. Congruent phylogenies obtained by analyses of tuf gene and ribosomal protein (rp) operon gene sequences further resolved the diversity among AY group phytoplasmas. Distinct phylogenetic lineages were identified by RFLP analysis of 16S rRNA, tuf or rp gene sequences. Ten subgroups were differentiated, based on analysis of rp gene sequences. It is proposed that AY group phytoplasmas represent at least one novel taxon. Strain OAY, which is a member of subgroups 16SrI-B, rpi-B and tuf-B and is associated with evening primrose (Oenothera hookeri) virescence in Michigan, USA, was selected as the reference strain for the novel taxon Candidatus Phytoplasma asteris'. A comprehensive database of diverse AY phytoplasma strains and their geographical distribution is presented.

http://ijs.sgmjournals.org/cgi/content/abstract/53/6/1725

The chloroplast genes of dinoflagellates are distributed among small, circular dsDNA molecules termed minicircles. In this paper, we describe the structure of the non-coding region of the psbA minicircle from Symbiodinium. DNA sequence was obtained from five Symbiodinium strains obtained from four different coral host species (Goniopora tenuidens, Heliofungia actiniformis, Leptastrea purpurea and Pocillopora damicornis), which had previously been determined to be closely related using LSU rDNA region D1/D2 sequence analysis. Eight distinct sequence blocks, consisting of four conserved cores interspersed with two metastable regions and flanked by two variable regions, occurred at similar positions in all strains. Inverted repeats (IRs) occurred in tandem or twin' formation within two of the four cores. The metastable regions also consisted of twin IRs and had modular behaviour, being either fully present or completely absent in the different strains. These twin IRs are similar in sequence to double-hairpin elements (DHEs) found in the mitochondrial genomes of some fungi, and may be mobile elements or may serve a functional role in recombination or replication. Within the central unit (consisting of the cores plus the metastable regions), all IRs contained perfect sequence inverses, implying they are highly evolved. IRs were also present outside the central unit but these were imperfect and possessed by individual strains only. A central adenine-rich sequence most closely resembled one in the centre of the non-coding part of Amphidinium operculatum minicircles, and is a potential origin of replication. Sequence polymorphism was extremely high in the variable regions, suggesting that these regions may be useful for distinguishing strains that cannot be differentiated using molecular markers currently available for Symbiodinium.


http://ijs.sgmjournals.org/cgi/content/abstract/54/4/1063

A moderately thermophilic and alkaliphilic bacillus, which had been reported and designated BLx (Haruta et al., 2002), was isolated from a semi-continuous decomposing system of kitchen refuse. Cells of strain BLxT were strictly aerobic, rod-shaped, motile and spore forming. The optimum temperature and pH for growth were approximately 50{degrees}C and pH 8-9. Strain BLxT was able to grow at NaCl concentrations from 0{middle dot}5 to 7{middle dot}5 %, with optimum growth at 0{middle dot}5 % NaCl. The predominant menaquinone was MK-7, and the major fatty acid was iso-C15: 0. Phylogenetic analysis showed that strain BLxT was positioned in an independent lineage within the cluster that includes the genera Virgibacillus and Lentibacillus in Bacillus rRNA group 1. Strain BLxT exhibited 16S rDNA similarity of 92{middle dot}8-94{middle dot}4 % to Virgibacillus species and 92{middle dot}3 % to Lentibacillus salicampi. Phenotypic, chemotaxonomic and phylogenetic analyses supported the classification of strain BLxT in a novel genus and species. Cerasibacillus quisquiliarum gen. nov., sp. nov. is proposed on the basis of phenotypic, chemotaxonomic and phylogenetic data. The type strain is BLxT (DSM 15825T=IAM15044T=KCTC 3815T).

http://ijs.sgmjournals.org/cgi/content/abstract/53/6/2019

The family Vibrionaceae is considered to be one of the most diverse and well-studied groups of bacteria. Here, evolution is assessed within the Vibrionaceae to determine whether multiple origins of eukaryotic associations have occurred within this diverse group of bacteria. Analyses were based on a large molecular dataset, along with a matrix that consisted of 100 biochemical and restriction digest characters. By using direct optimization methods to analyse both datasets individually and in combination, a total-evidence cladogram has been produced, which supports the hypothesis that several important symbionts (both mutualistic and pathogenic) within the Vibrionaceae are not monophyletic. This leads us to consider that symbiosis (and subsequently, associations with Eukarya) has evolved multiple times within the Vibrionaceae lineage.


http://ijs.sgmjournals.org/cgi/content/abstract/54/3/759

Strain H2-LRT, a 5-18 {micro}m long and 0{middle dot}7 {micro}m wide filamentous, mesophilic, moderately halophilic, non-motile hydrogenotrophic methanogen, was isolated from marine sediment of Aarhus Bay, Denmark, 1{middle dot}7 m below the sediment surface. On the basis of 16S rRNA gene comparison with sequences of known methanogens, strain H2-LRT could be affiliated to the genus Methanobacterium. The strain forms a distinct line of descent within this genus, with Methanobacterium oryzae (95{middle dot}9 % sequence identity) and Methanobacterium bryantii (95{middle dot}7 % sequence identity) as its closest relatives. The 16S rRNA-based affiliation was supported by comparison of the mcrA gene, which encodes the {alpha}-subunit of methyl-coenzyme M reductase. Strain H2-LRT grew only on H2/CO2. The DNA G+C content is 34{middle dot}9 mol%. Optimum growth temperature was 45 {degrees}C. The strain grew equally well at pH 7{middle dot}0 and 8. No growth or methane production was observed below pH 5 or above pH 9. Strain H2-LRT grew well within an NaCl concentration range of 100 and 900 mM. No growth or methane production was observed at 1 M NaCl. At 50 mM NaCl, growth and methane production were reduced. Based on 16S rRNA gene sequence analysis, the isolate is proposed to represent a novel taxon within the genus Methanobacterium, namely Methanobacterium aarhusense sp. nov. The type strain is H2-LRT (=DSM 15219T=ATCC BAA-828T).


http://ijs.sgmjournals.org/cgi/content/abstract/53/2/455

Sequence analysis of rpoB, the gene encoding the{beta} -subunit of RNA polymerase, was used in a phylogenetic investigation of nine species from the genera Ehrlichia, Neorickettsia, Wolbachia and Anaplasma. The complete nucleotide sequences obtained for Anaplasma phagocytophilum (HGE agent), Ehrlichia chaffeensis, Neorickettsia sennetsu, Neorickettsia risticii, Anaplasma marginale and Wolbachia pipientis were amongst the longest rpoB sequences in GenBank and ranged from 4074 bp for N. sennetsu to 4311 bp for W. pipientis. Additional
partial rpoB sequences were obtained for Ehrlichia canis, Ehrlichia ruminantium and Ehrlichia muris. Identical phylogenetic trees were inferred from multiple sequence alignments of the nucleotide sequences and the derived amino acid sequences using either distance, maximum-likelihood or parsimony methods. This study confirms the phylogeny previously inferred from sequence analyses of the 16S rRNA gene, groESL and gltA and allows the confirmation of four monophyletic clades. The rpoB nucleotide sequences were more variable than the 16S rRNA gene and groESL sequences at the species level.


This study analysed the usefulness of recA gene sequences as an alternative phylogenetic and/or identification marker for vibrios. The recA sequences suggest that the genus Vibrio is polyphyletic. The high heterogeneity observed within vibrios was congruent with former polyphasic taxonomic studies on this group. Photobacterium species clustered together and apparently nested within vibrios, while Grimontia hollisae was apart from other vibrios. Within the vibrios, Vibrio cholerae and Vibrio mimicus clustered apart from the other genus members. Vibrio harveyi- and Vibrio splendidus-related species formed compact separated groups. On the other hand, species related to Vibrio tubiashii appeared scattered in the phylogenetic tree. The pairs Vibrio corallilyticus and Vibrio neptunius, Vibrio nereis and Vibrio xuii and V. tubiashii and Vibrio briansiensis clustered completely apart from each other. There was a correlation of 0.58 between recA and 16S rDNA pairwise similarities. Strains of the same species have at least 94 % recA sequence similarity. recA gene sequences are much more discriminatory than 16S rDNA. For 16S rDNA similarity values above 98 % there was a wide range of recA similarities, from 83 to 99 %.


We studied the expression of a human macrophage lectin specific for galactose/N-acetylgalactosamine (hMGL) during macrophage differentiation. The expression of hMGL during the in vitro differentiation induced by human serum was examined by immunostaining and Western blotting with a specific mAb, MLD-1, as well as with RT-PCR analysis. hMGL was detected on cells at an intermediate stage of differentiation. These cells were round, slightly larger in size (12.7 +/- 0.2 {micro}m) than monocytes (9.8 +/- 0.1 {micro}m) and expressed the macrophage marker CD14, but lacked the dendritic cell marker CD1a. The highest levels of expression occurred after 2-4 days of culture. At this time point, MLD-1 prominently stained 20-40% of the cells. Monocytes cultured for 16 h or fully differentiated monocyte-derived macrophages were negative or weak for hMGL expression. Similar transient expression was also observed during granulocyte macrophage colony stimulating factor- or macrophage colony...
stimulating factor-dependent macrophage differentiation. The lectin was characterized as a functional endocytic receptor for glycosylated macromolecules, since the uptake of carbohydrate polymers was partially inhibited by the addition of MLD-1. The distribution of hMGL+ cells in normal human skin was found by immunostaining to be mainly in the upper dermis distant from vascular structures. More than 90% of the hMGL+ cells were double stained with anti-CD68 mAb and constituted [~]20% of the CD68+ cells. We suggest that the dermal hMGL+ cells are a subset of differentiated cells derived from monocytes and that hMGL is a unique marker for cells at an intermediate stage of macrophage differentiation.


http://intimm.oupjournals.org/cgi/content/abstract/15/8/1017

The vitamin A metabolite, retinoic acid (RA), affects Th1 and Th2 development. The effect is partly exerted through the modulation of antigen-presenting cell functions, but it remains unclear whether RA directly exerts its effect on T cells to influence Th1/Th2 development. To clarify this problem, we used two experimental systems with isolated T cells in vitro. In one system, isolated CD4+CD8+ thymocytes differentiated into Th1 and Th2 by two transient stimulations with defined combinations of ionomycin and phorbol myristate acetate followed by treatment with IL-2 and IL-4 and/or IL-12. In the second system, functional differentiation was induced in purified naive CD4 T cells from DO-11.10 TCR-transgenic and RAG-2-deficient mice with cytokines and antibodies to CD3 and CD28. In both systems, all-trans-RA at [IMG]=" BORDER="0">1 nM concentrations suppressed Th1 development, but enhanced Th2 development. 9-cis-RA elicited similar effects. The optimal enhancement of Th2 development in the second system, however, was achieved with a delayed addition of RA. The presence of RA during the initial stimulation period often suppressed Th2 development. The RA receptor (RAR) antagonists, LE540 and LE135, but not the retinoic X receptor (RXR) antagonist, PA452, inhibited the effect of RA on Th1/Th2 development. Accordingly, the RAR agonists, Am80 and Tp80, but not the RXR agonists, HX600 and TZ335, mimicked the effect of RA. The RXR agonists enhanced the effect of the RAR agonists only slightly, if at all. These results indicate that, via RAR, RA directly suppresses Th1 development and directly enhances Th2 development with its timely addition.


http://intimm.oupjournals.org/cgi/content/abstract/14/1/79

We have previously identified a locus on mouse chromosome 15 (eae2) that regulates susceptibility to experimental autoimmune encephalomyelitis in a cross between the susceptible strain B10.RIII and the resistant strain RRIS/J. In an effort to verify the protective effect from having two RRIS/J alleles at eae2, the resistant locus was bred into the susceptible strain in homozygous form. However, the expected effect was not as clear as in the original study. This might be due to an epistatic effect conferred by several unidentified genes in the genome of the resistant strain or due to the environment by genotype interactions, possibly overcoming the effect of protective alleles at eae2. To further the genetic understanding in this disease, a genome-wide linkage screening approach was employed on an F2 intercross that carried the protective allele at eae2 in homozygous form while the rest of the genome segregated between the B10.RIII and RRIS/J strains as in the original investigation. In the present study we find one region on chromosome 7, not previously identified in this strain combination, that affects the
disease at significant logarithm of the odds score and six regions showing suggestive evidence for linkage to disease phenotypes.


http://intimm.oupjournals.org/cgi/content/abstract/15/1/109

Mannose-binding lectin (MBL) is a C-type lectin involved in the first line of host defense and it requires MBL-associated serine proteases (MASP) for activation of the lectin complement pathway (LCP). Recently we reported that human ficolins, L-ficolin/P35 and H-ficolin/Hakata antigen, as well as MBL activate the LCP in association with MASP. We investigated in vitro expression of complements of the lectin complement pathway in several cell lines. Out of 17 cell lines tested using RT-PCR, a human glioma cell line, T98G, expressed high levels of H-ficolin/Hakata antigen, MASP1 and MASP3 mRNAs. Similar results were obtained in four other glioma lines. In addition, mRNAs for C1r, C1s, C2, C3, C4, C5 and C6 were also detected in T98G cells, but very low amount of mRNAs for C1q and MBL. MBL mRNA was seen in two of the other glioma cell lines. An ELISA of culture supernatants showed that T98G cells secreted a considerable amount of MASP-1 and MASP-3 proteins. SDS-PAGE and immunoblotting analyses showed the secreted H-ficolin/Hakata antigen, MASP-1 and MASP-3 to be 34, 81 and 105 kDa in size respectively, similar to their serum counterparts. Since the glioma cells used are derived from astrocytes, this suggests that human astrocytes may be a source of some components of the LCP in the brain.


http://intimm.oupjournals.org/cgi/content/abstract/14/10/1203

Maturation of dendritic cells (DC) serves a deterministic role in the link between innate and adaptive immunity, constituting a checkpoint with regard to whether responses from the lymphocyte compartment shall be raised and what class of response is needed to protect the host against invading pathogens. Since DC have not been shown to possess mechanisms such as gene recombination or somatic mutation for generating a diverse repertoire of antigen-recognition receptors, it is unlikely that these leukocytes can intrinsically respond to all conceivable molecules present in our environment. In the present study, we have therefore determined how mediators of the inflammatory response regulate global gene transcription in DC. The data represent an extensive and time-ordered reprogramming of the DC during their course of maturation, involving genes encoding proteins that regulate responses of both innate cells and lymphocytes. This transcriptional reorganization may reflect the effect of in vivo released inflammatory mediators induced by endogenous or pathogenic stimulation.


http://intimm.oupjournals.org/cgi/content/abstract/16/1/91
Peyer's patch follicle-associated epithelium (FAE) regulates intestinal antigen access to the immune system in part through the action of microfold (M) cells which mediate transcytosis of antigens and microorganisms. Studies on M cells have been limited by the difficulties in isolating purified cells, so we applied TOGA mRNA expression profiling to identify genes associated with the in vitro induction of M cell-like features in Caco-2 cells and tested them against normal Peyer's patch tissue for their expression in FAE. Among the genes identified by this method, laminin (beta)3, a matrix metalloproteinase and a tetraspan family member, showed enriched expression in FAE of mouse Peyer's patches. Moreover, the C. perfringens enterotoxin receptor (CPE-R) appeared to be expressed more strongly by UEA-1+ M cells relative to neighboring FAE. Expression of the tetraspan TM4SF3 gene and CPE-R was also confirmed in human Peyer's patch FAE. Our results suggest that while the Caco-2 differentiation model is associated with some functional features of M cells, the genes induced may instead reflect the acquisition of a more general FAE phenotype, sharing only select features with the M cell subset.


http://intimm.oupjournals.org/cgi/content/abstract/14/5/481

MHC class II expression defects have been evidenced in several human tumor cell lines originating from lung cancers or retinoblastoma. Accordingly, the mouse adenocarcinoma and fibrosarcoma cell lines, RAG and L(tk-), do not express I-A and I-E molecules even when treated with IFN-γ. Here we show that fusion of both cell lines restores the inducible expression of MHC class II, thereby demonstrating that they present different and recessive alterations outside the MHC class II locus. CIITA, the MHC class II transactivator, controls the tissue-specific expression of MHC class II genes and creates the architecture of the transcriptional complex that binds to the MHC class II gene promoters. In L(tk-) cells, C2ta transcripts, expressed from the gene encoding CIITA, were indeed detected in severely limited amounts, with a defect in C2ta transcription initiation. In agreement we show here that the L(tk-) cell line does not express the CIITA protein. In contrast, in the RAG cell line, C2ta transcripts were expressed at normal levels, from the proper initiation site. The nucleotide sequencing of the CIITA cDNA from RAG did not reveal any mutation. However, the CIITA protein was not detected. These data evidence a new type of defect in a MHC class II-defective tumor cell line, as we show here that the alteration in the RAG cells occurs downstream of C2ta transcription. The RAG mutation might therefore reside in the C2ta transcript nuclear export or translation, or in the stability of the CIITA protein.


http://intimm.oupjournals.org/cgi/content/abstract/14/10/1085

Cytotoxic lymphocytes, NK cells and CD8+ T cells play a pivotal role in the host defense. To reveal the biological function of these cells through establishing a comprehensive gene expression profile, serial analysis of gene expression was performed in human peripheral blood NK cells and CD8+ T cells. In total, 85,848 tags corresponding to >20,000 different transcripts were sequenced. The genes expressed abundantly in these libraries mostly consisted of genes encoding MHC class I and molecules related to protein synthesis. Among gene transcripts which related to cytotoxicity, granulysin, perforin, granzyme B and (alpha)-defensin 1 were highly expressed in NK cells. Resting CD8+ T cells did not express the genes related to cytotoxicity, but expressed abundantly the genes encoding chemokines, tumor necrosis factor family. When CD8+ T cells were sorted into naive, memory and effector subsets based on the expression of CD45RA and CD27, perforin and granzyme B were expressed in the CD45RA+CD27- effector
subset. (alpha)-Defensin 1, one of the selectively expressed genes in NK cells, induced migration of naive CD8+CD45RA+CD27+ T cells, but not memory CD8+CD45RA-CD27+ or effector CD8+CD45RA+CD27- T cells. Furthermore, treatment with IL-15, a stimulator of NK cell development, differentiation, survival and cytotoxicity, rapidly enhanced the expression of (alpha)-defensin 1 in NK cells. The identification of the genes preferentially expressed in NK and CD8+ T cell subsets may give important insights into the functions of these cells against virus infection and in tumor immunity.


http://intimm.oupjournals.org/cgi/content/abstract/14/6/599

In order to explore the role of gp130-linked signal transduction in the differentiation and maturation of dendritic cells (DC), the mAb, B-S12, an agonist of gp130, was used for the activation of gp130 on DC. The effects of cytokines and of anti-gp130 mAb on the proliferation of DC, and their expression of IL-12 and CD80 (B7-1) by DC were evaluated. DC differentiating from peripheral blood mononuclear cells did not express the IL-6 receptor (alpha) chain, but expressed gp130. Anti-gp130 mAb promoted the proliferation of DC, induced by IL-4 and granulocyte macrophage colony stimulating factor (GM-CSF), by up-regulating the GM-CSF receptor on DC. DC induced by gp130 mAb and cytokines expressed DC-derived CC chemokine, as measured by RT-PCR. Induced DC also stimulated strong proliferation of autologous T cells in mixed lymphocyte reaction since an up-regulated expression of IL-12 and CD80 (B7-1) was observed in DC activated by anti-gp130 mAb. Thus, gp130 signal transduction is important for the differentiation and maturation of DC.


http://intimm.oupjournals.org/cgi/content/abstract/15/9/1073

T cells recognizing myelin basic protein (MBP) are potentially involved in the pathogenesis of multiple sclerosis (MS). In vivo clonal expansion of MBP-reactive T cells in MS may relate in part to dysfunction of peripheral regulatory mechanisms, including the anti-idiotypic immune network. In this study, we examined anti-idiotypic immune responses and the functional properties of anti-idiotypic T cells in patients with MS and healthy controls using TCR peptides corresponding to a CDR3 sequence motif preferentially expressed among T cells recognizing the 83-99 immunodominant peptide of MBP in some patients with MS. The study demonstrated that anti-idiotypic T cells could be induced in vitro by 8mer and 15mer peptides containing the CDR3 motif in MS patients and healthy controls respectively. The estimated precursor frequency of the anti-idiotypic T cells was slightly reduced in MS patients compared to control subjects. The obtained anti-idiotypic T cells recognizing the 15mer TCR peptide were found to express the CD4 phenotype, produce predominantly IL-10 and inhibit the proliferation of autologous T cells recognizing the immunodominant peptide of MBP. Anti-idiotypic T cells induced by the 8mer TCR peptide were predominantly CD8+ cytotoxic T cells and exhibited cytotoxic activity against autologous MBP-specific T cells expressing the CDR3 sequence. When added in primary culture, both TCR peptides had a significant inhibitory effect on the T cell responses to the immunodominant peptide of MBP. The findings suggest that anti-idiotypic immune responses can be activated by selected TCR peptides and may play an important role in the in vivo regulation of MBP-reactive T cells.

http://www.sciencedirect.com/science/article/B6VG6-4D4D2PX-2/2/50410d2debb8ee8476d49d6a8e9b88358

Explorers to Antarctica during the Heroic Era of exploration built three wooden huts on Ross Island, Antarctica in 1902, 1908 and 1911. The structures were used as bases of operation while their occupants participated in scientific endeavors and strived to reach the South Pole. The huts, and the thousands of artifacts in and around them, have survived in the Antarctic environment for 9-10 decades, but deterioration has taken place. The successful preservation of these important historic structures and materials requires information on the agents causing deterioration and factors that influence microbial growth. Temperature and relative humidity (RH) were monitored in the expedition huts for several years. During the austral summer months of December and January it was common for temperatures to rise above and RH to exceed 80%. Extensive fungal growth was observed on wood and artifacts within the Cape Evans hut, and fungi isolated were identified as species of Cladosporium, Penicillium, Cadophora, Geomyces and Hormonema. The factors that influence RH within the huts and methods to control moisture and arrest microbial growth are discussed.


http://www.sciencedirect.com/science/article/B6VG6-47X1X1M-F/2/3b076c9ecee4d61e0ed31647068ad617

Preliminary results on limestone weathering caused by air pollution and microbial colonization are presented in this study. Outdoor exposure experimental assays were performed on Scaglia limestone samples. Samples were exposed in two areas in Perugia (Italy) that differ in degree of urban air pollution. At different times of exposure, ranging from 1 to 12 months, microbial contamination of sampled surfaces was evaluated by microbiological techniques, genotyping and scanning electron microscopy. After 1 year of exposure, a significant fungal colonization and the presence of weathering products (i.e., gypsum) were detected on sampled surfaces.

In this study, we have analysed the nucleotide sequence variation of the 12S rRNA mitochondrial gene (648-1601 bp) from five different populations (Spanish Caucasian, Autochthonous from the Basque Country, Chinese, New Guinea Highlander and Africans of Benin) by full sequencing of two overlapping PCR fragments using d-rhodamine cycle sequencing coupled with an ABI377 sequencer. Preliminary data indicate different patterns of sequence variation between Africans and non-Africans. Africans are much more polymorphic than non-African populations, which have only a very restricted subset of haplotypes. Furthermore, the greater part of Africans analysed showed two specific nucleotide substitutions (769G->A and 1018G->A) that were not observed in non-African individuals. In conclusion, the mtDNA 12SRNA gene in combination with other systems could be an interesting ethnic marker that could help to differentiate between African and non-African maternal lineages.


Our casuistic in paternity testing showed several isolated incompatibilities present in different STRs like FES, SE33, vWA, among others, include homozygote and heterozygote situations, and appear both in father-child and mother-child pairs. As we assume, in paternity testing, that the mother is the real one, most of incompatibility situations are assigned to a mutation in putative father. In fact, only sequencing allows the real explanation for the event.


We present the optimisation of two Y-STR multiplexes for forensic casework applications especially for the analysis of mixtures of male and female DNA. The procedure involved: (1) a new design of the PCR primers for the loci DYS389, DYS390 and DYS391 in order to improve the PCR efficiency and to reduce the length of the amplification products, and (2) the addition of PCR Enhancer to the reaction mix, increasing the specificity of the method.

A heptaplex PCR has been developed to amplify DXS6789, HUMARA, DXS10011, DXS7423, HPRTB, DXS6807 and DXS101 on Italian samples from Bologna, Modena, Padova, Ancona and Pisa. Statistical analyses were performed for all the loci.


Background: The polymorphism of the two hypervariable segments (HVI and HVII) of the control region of mtDNA was analyzed in a population of 81 unrelated individuals from Central Portugal and 48 from the Azores Islands, using a fluorescent-based electrophoresis sequencing method.

Methods: Sequences have been obtained with ABI PRISM0 Big Dye Terminator and dRhodamine Terminator Cycle Sequencing Ready Reaction Kits, with Amplitaq DNA Polymerase FS, and have been detected with ABI PRISM 377 DNA sequencer. Results: In the Central Portugal population (n=81), we observed 69 polymorphic sites of sequence in HVI region and 44 in HVII region. In the Azores population (n=48), we observed 48 polymorphic sites of sequence in HVI region and 24 in HVII region. Conclusions: Nucleotide substitution rather than insertion/deletion (1 or 2 bp) was the majority of variation. The distribution showed a large bias towards transitional changes than transversional changes. Our sequencing results are similar to other Caucasian population data.


Denaturing high-performance liquid chromatography (dHPLC) is a new automated sizing method when used in non-denaturing conditions and is a powerful mutation detection tool in denaturing conditions. We introduce three possible forensic applications of this new technology. (1) Determination of age at death. Various mutations accumulate in mtDNA during ageing. According to this, we are developing a new method to determine age at death based on dHPLC ability to detect mtDNA mutations. mtDNA is extracted from autopsy tissues (iliopsoas, liver, kidney, putamen and heart) of numerous individuals representing a wide age spectrum. After amplification and digestion of the entire mtDNA in 90-600 bp fragments, separation is performed by dHPLC at different temperatures. We are studying the qualitative and quantitative differential accumulation of mutations with age among the various tissues. Our methodology is presented. (2) Human identification. In non-denaturing conditions, dHPLC gives size-based separation of DNA fragments. This allows separation of short tandem repeats (STRs) fragments. The different alleles of the HUMTH01, F13A01, vWa31 and FESFPS loci were separated and sized in 5 min. (3) Gender determination. Used in non-denaturing conditions, dHPLC can separate the two sex-specific alleles of the amelogenin locus in 4 min without any preparation of the PCR product.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-15/2/a0118765706b57bdc0c0193d9e9f68fc

A multiplex-PCR procedure was developed for the analysis of single nucleotide polymorphism (SNP) variation defining the European mtDNA-haplotypes in the coding part of the mitochondrial genome. The obtained PCR-products were genotyped for 26 SNPs in four mini-sequencing (SNaPshot(TM)) reactions and analyzed on the ABI PRISM(R) 3100 Genetic Analyzer. A population study of 157 individuals of Belgian descent revealed 25 different haplotypes belonging to 5 major haplogroups.


http://www.sciencedirect.com/science/article/B7581-47W664D-1H2/2/718a3750b0a508a4a07961059417b621

We present the frequency distributions of 13 Y-specific STR polymorphisms (DYS19, DXYS156, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 and YCAII) and the frequency of the combination of these haplotypes in Vietnamese males.


http://www.sciencedirect.com/science/article/B7581-47W60JD-4X2/d71c9a20c7930e85b9294a30fdef8264

Plasmid-driven synthesis of viral RNA and protein allows the recovery of infectious influenza virus without the need for helper virus infection. Because no selection system is required for this approach, genetic manipulation of all eight viral gene segments without technical limitations is possible. We have developed a system which requires the construction and transfection of only eight plasmids for the recovery of influenza A viruses. In this DNA transfection system, viral cDNA is inserted between the human RNA polymerase I (pol I) promoter and murine terminator sequences. The entire pol I transcription unit is flanked by an RNA polymerase II (pol II) promoter and a poly(A) site. As a first step to evaluate the utility of this plasmid-based system for the production of vaccines, we generated the master strain A/PR/8/34 (H1N1) currently used for the production of inactivated vaccines entirely from cloned cDNAs. The virus yield as determined by HA-assay after passage of the recombinant virus in eggs was as high as the virus yield of the parental wild-type virus. These results prove that the generated recombinant virus has the same growth properties as the parental egg grown virus and indicate that the eight-plasmid transfection method has the potential to improve currently used methods for the production of vaccine viruses.


In this validation study, we have evaluated the efficacy and the validity of the SGM Plus test using an amplification regime of 34 cycles. We obtained valid DNA typing results from pristine extracts with an extremely low DNA content. In this context, the aspects of single cell PCR typing were also evaluated. In these experiments, the allele dropout phenomenon was clearly demonstrated. From actual casework samples, we obtained conclusive DNA profiles from highly purified extracts of bone and teeth that failed to demonstrate typing results using the standard PCR protocol of 28 cycles. Moreover, low copy number (LCN) DNA typing offered us the possibility to reanalyse crime samples that failed to produce a conclusive profile after 28 cycles. Unfortunately, several complications accompany ultrasensitive PCR amplification. During our validation studies, we have observed increased risk of contamination, allelic dropout, locus dropout and heightened stutters. Analyses of heterozygote balance, between-loci balance and stutter heights, show that the 34-cycle PCR has its own characteristic features. We finally show that reamplification of SGM Plus PCR products by an extra 6 PCR cycles offers a promising new alternative if too little of the original sample extract is left for a complete reanalysis.


We mapped human STS UT413 on human chromosome Xq27-q28, renamed it DXS10011 and developed an easy method of analysis using capillary electrophoresis. The probability of discrimination was 0.954 from 1198 chromosomes in the Japanese population. DXS10011 is a hypervariable and stable marker on the human X chromosome.


Background: The hemagglutination remains crucial in diagnosis of influenza virus and for the antigenic characterization of the hemagglutinin (HA) and neuraminidase. However, the human influenza viruses A(H3N2) isolated recently appear to have lost the ability to agglutinate chicken erythrocytes (ER) (RBC). The molecular determinants of this phenomenon are not known. Methods: Two viruses isolated in Paris were studied, since their ability to agglutinate chicken RBC was observed after serial passages either in Madin Darby canine kidney (MDCK) cells or embryonated hen's eggs. Sequencing analyses and hemadsorption assays were performed to demonstrate the role of amino acid substitutions in the HA gene. Results: Sequencing of the HA
gene revealed the presence of either the Leu194Ile or the Val226Ile mutation following the phenotypic change. Hemadsorption assays performed following transfection of COS-1 cells, with plasmids expressing wild-type or mutated HA molecules, showed that the Leu194Ile and Val226Ile mutations were responsible for the ability of the HA to bind chicken RBCs. Conclusion: These findings suggest that a valine at position 226 in the HA molecule, found in recent clinical isolates of human A(H3N2) viruses, could be responsible for their inability to agglutinate chicken erythrocytes.


http://www.sciencedirect.com/science/article/B7581-47W664D-1DN/2/708647627c0adb225adac1051e3fd3f8

We developed the allele-specific TaqMan polymerase chain reaction (AS-TaqMan PCR) and SYBR Green PCR assays for detecting glycophorin A (GYP A), low-density lipoprotein receptor (LD LR), hemoglobin G (HBGG), D7S8 and group-specific component (GC) alleles. We improved the specificity of detecting a nucleotide substitution by introducing the additional mismatches at position 2 (3 in GYP A). The differences between threshold cycle (Ct) values of different genotypes on each of the loci were statistically significantly different. All the genotypes agreed with the results using the AmpliType PM+DQA1 PCR Amplification and Typing kit. The AS-TaqMan PCR and SYBR Green PCR assays are simple, rapid, and accurate, as well as suitable for high-throughput applications in a forensic investigation.


http://www.sciencedirect.com/science/article/B7581-47W664D-1NT/2/c4b0cafebdadc36c6a16f7ea26b082a1


http://www.sciencedirect.com/science/article/B7581-4C4WDDP-2S/2/8e9f10c4a62a8e7d5a2250f5fab6d93a

The tetranucleotide repeat loci D6S389 and D6S1051 situated nearby the HLA class II region (6p) were investigated in an Austrian Caucasian population sample. Typing of the amplification products and cycle sequencing were carried out using denaturing capillary electrophoresis. For D6S389 and D6S1051, 19 and 7 different alleles were observed. Sequencing of D6S389 revealed a (GAAA)n repeat pattern in the common alleles. Additionally, infrequent "1" alleles were seen. Alleles of D6S1051 showed a simple (GATA)n repeat structure and an A/G-SNP next to the repeat region as well as a T/C-SNP in the 3’-flanking region. D6S389 proved to be a highly polymorphic marker. D6S1051 is less polymorphic, but interesting because of the existing SNPs.

During the last few years, there is an increasing interest in the use of Single Nucleotide Polymorphisms (SNPs) for forensic purposes as an alternative to STR analysis. At this moment, development of SNP genotyping technologies to analyse several markers in the same reaction with high accuracy, simplicity and reasonable cost is the key to progress in SNP typing for forensic genetics. A promising approach for this purpose is DNA microarrays. We have developed a microarray for typing a set of 29 Y-chromosome SNPs for European populations. Single Base Extension and Tags (SBE-Tag) has been the strategy selected.


The STR locus C_2_4_4 situated in the HLA class I region (6p21.3) was investigated in an Austrian Causasoid population sample of 247 unrelated individuals. Beside length polymorphism, the alleles at this locus also revealed sequence polymorphism.


We selected the DYS19, DYS385, DYS389, DYS390, DYS392, DYS393, DYS483, and amelogenin loci, and designed a new pair of primers to minimize the fragment sizes of these loci as much as possible. As a consequence, these loci were able to detect in the range of 79-259 bp using multiplex PCR amplification. The optimum DNA amount was 100 pg to 10 ng. The haplotype diversity was 0.9979.


We performed multiplex PCR for the TH01, TPOX, CSF1PO, and vWA loci using newly designed pairs of primers that yield smaller fragments than previously reported [Int. J. Leg. Med. 114 (2001) 285; Am. J. Hum. Genet. 55 (1994) 175; Int. J. Leg. Med. 106 (1994) 183.][1, 2 and 3]. This system required genomic DNA in a range of 50 pg-2 ng, and proved to be sensitive as a typing method. Furthermore, it was possible to determine the allele types even from 18-year-old bloodstains.
Population genetic data of three tetranucleotide X-chromosomal STRs, DXS7132, DXS7133 and GATA172D05, were obtained by analyzing 295 unrelated healthy individuals living in North and South Italy (160 females and 135 males), and 40 family trios with female children. PCR primers for the loci DXS7132 and DXS7133 were redesigned in order to reduce the length of the amplification products compared with conventional design so that improved typing success rate when highly degraded DNA is used as a template. The comparison of the allele frequencies of these three ChrX markers gave similar distributions for North and South Italy although minor variations were found for some alleles. Additionally, some differences were found when comparing the allele frequencies of the male and female samples independently. Based on the investigated meiotic events, mutations were not found.

A procedure was developed for genotyping one CYP2C19 and eight CYP2D6 polymorphisms. After multiplex PCR of the exons containing the SNPs, a mini-sequencing reaction was performed with the SNapShot(TM) Multiplex Kit and the resultant primer-extension products were analyzed on an ABI PRISM(R) 3100 Genetic Analyzer. The developed procedure was validated in a population of 199 Caucasians.

Degraded samples were studied in order to obtain Y-STR haplotype to provide additional data in paternal lineage identification caseworks. Multiplex reactions were used comprising the minimal Y-STR haplotype DYS19, DYS390, DYS391, DYS392, DYS393, DYS19, DYS389I/II and DYS385 belonging to the Y-STR database. The other Y-STR loci--GATA A 7.1, GATA A 7.2, GATA C4, GATA H4, DYS437, DYS438 and DYS439--were included in the Y-Chromosome Quality Control Group of the Spanish and Portuguese Group (GEPY) of the ISFG. Y-STRs results were successful in almost all the samples when applied some modifications in amplification methods.

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In this study, we investigated the short tandem repeat loci C1_4_4, C2_4_4 and C3_3_6 situated in the HLA class I region in a sample of 153 unrelated Austrian Caucasoid individuals by multiplex PCR. The sequence structure of C1_4_4 and C3_3_6 alleles is described, as well as the sequence of a new C2_4_4 allele, allele 23.


http://www.sciencedirect.com/science/article/B7581-47W60JD-13/2/3c6ae1f0b0f18c608626b592c549cf834

Background: Since Taiwan has high population density and a similar ecological environment to Mainland China, the epi-center of influenza viruses, it is very important to establish influenza virologic surveillance systems in both animal and human populations. The H5N1 Hong Kong Flu in 1997 and H9N2 in 1999 have showed that avian influenza viruses can cross the receptor of host species boundary and transmit to human. Therefore, the specific aim of this study is to understand the frequency of inter-species transmission in Taiwan. Methods: We have established an avian influenza virologic surveillance system in one of the largest live poultry markets in Taipei City from October 1999 to March 2000. Serum samples were collected from 341 blood donors, including veterinarians, poultry farm workers, and market employees. HI and microneutralization were used to detect specific antibody against H6, an endemic virus in chicken farms in southern Taiwan, and antibodies against H3, H4, and H9 viruses. Results: Among about 1300 fecal specimens of chickens and ducks collected, we isolated 12 H3 viruses, 14 H4 viruses, and 2 H6 viruses (i.e. 9 serotypes of HA and NA) from ducks. The isolation rates were 0% (0/580) and 7% (28/400) in chickens and ducks, respectively. Phylogenetic analysis of HA from 7 of our 12 H3 isolates showed the highest (93%) homology with A/equine/Jilin/89 (H3N8). Both phylogenetic relationship of HA and NP genes from selected representative strains (7 H3, 7 H4, and 2 H6) found they all fell into Eurasian lineage of avian influenza viruses. Their NP genes were away from the G1 lineage that was found in H5N1 strain isolated in Hong Kong in 1997. In addition, the results of HI and microneutralization tests found that they were all seronegative against two avian influenza virus strains [A/Duck/Czechoslovakia/56 (H4N6) and A/Shearwater/Australia/1/72 (H6N5)]. Conclusion: Continuous efforts by integrating animal, market and human influenza surveillance systems have provided the best early warning signals to detect new influenza virus activities for preventing potential pandemics and providing effective controls.


http://www.sciencedirect.com/science/article/B7581-47W664D-1K6/2/bd3ca57048b84a0f2c2186bc984260ad

Based on the method described in Stone et al.'s [Am. J. Phys. Anthropol. 99 (1999) 231] publication, a fragment of the amelogenin gene (exon 6) was shortened. In a new method, a flanked and amplified 78-bp fragment from X and Y copies of the amelogenin gene, followed by hybridization and ligation with fluorescent-labeled oligonucleotides, resulted in two gender-specific products analyzed by capillary electrophoresis. The method was tested using fresh DNA samples and DNA isolated from bones of different ages.

http://www.sciencedirect.com/science/article/B6T7C-47VS61M-1/2/346576973b671ab137397d3a96e4b516

A multiplex PCR able to identify cows’, goats’ and sheep’s milk in dairy products was developed. Specific primers were designed in the mitochondrial 12s and 16s rRNA genes so as to generate fragments of different length. The assay was applied to 19 cheeses from the retail trade in order to verify the label statements. The multiplex PCR results were confirmed by PCR-restriction fragment length polymorphism. The proposed multiplex PCR represents a rapid and sensitive method applicable on a routine basis. In fact it enables to detect, in a single step, goats’, sheep’s and cows’ milk in dairy products with a good sensitivity threshold (0.5%).


http://www.sciencedirect.com/science/article/B6T7C-4D2XFXX-1/2/611c7b6d43f638bf46e1e1ad94db9c95

The aim of this research was to elucidate the potential technological role of Streptococcus macedonicus in cheese. For this purpose, phenotypic and randomly amplified polymorphic DNA (RAPD)-PCR subtyping as well as biochemical characterization were carried out on 37 S. macedonicus isolates collected from several artisanal, Italian raw milk cheeses. Identification was achieved by a species-specific PCR assay developed in this study. A wide phenotypic and genotypic heterogeneity between the isolates was observed. A certain variability of the acidifying and peptidase activities was observed and the comparison between some technological characteristics displayed by S. macedonicus and Streptococcus thermophilus indicated a clear distinction of these species. The presence of dairy grade characteristics, such as peptidase activities, ability to produce inhibitory compounds, absence of antibiotic resistance and haemolytic activity, may suggest the rational design of a novel starter culture for dairy application composed of strains of both S. macedonicus and S. thermophilus species, with the former used as a starter adjunct.


http://www.sciencedirect.com/science/article/B6T7C-4CDJHKR-1/2/9463937d67a2442ab2e6fe99e6f999f0

The potential of a mass spectrometry (MS) based electronic nose to screen lactic acid bacteria producing volatile compounds was assessed. Thirty four reference strains and 62 Lactobacillus casei strains isolated from 5 dairies producing Gruyere cheeses were analysed. These isolates were classified into 7 different genotypes by Repetitive extragenic palindromic Polymerase chain reaction (REP-PCR). The strains were incubated in Ultra High Temperature milk supplemented
with casamino acids. After 10 days of incubation, the volatile fractions were analysed with the electronic nose. The strains with the same genotype were grouped together. The classification of strains based on the production of volatile compounds was in conformity with the classification obtained with the molecular method. This MS-based electronic nose can be used to differentiate bacterial populations in cheese samples and to screen for new aroma-producing strains.


http://www.sciencedirect.com/science/article/B6T7C-44GF0GD-2/2/0f9c3e16c35eed45432d6876018863e

The objective of our study was to investigate whether certain regions in the rpoD gene of Salmonella were suited for the RT-PCR detection of viable Salmonella cells. We performed RT-PCR on RNA extracted from viable, and heat- or ethanol-killed Salmonella cells. Using RT-PCR, RNA was easily detected in viable cells. For the heat-killed cells mRNA was undetectable after 1 h when stored at room temperature or 4[deg]C. For the ethanol-killed cells mRNA was detectable till 1 and 48 h after treatment, when the dead Salmonella cells were stored at room temperature and 4[deg]C, respectively. When the ethanol-killed cells that were stored at 4[deg]C, were incubated at 37[deg]C for 1 h prior to RNA extraction, no mRNA was detected. The primer pair used (salmrpd2/5) was directed to the housekeeping gene rpoD and using RT-PCR, signals from Salmonella were obtained and no cross-reaction was observed with other members of the Enterobacteriaceae.

*International Hepatology Communications* (2)


http://www.sciencedirect.com/science/article/B6T7D-4F6DWMP-3S/2/80e313b577a18c72574340ee9ca6f671

We determined quantitative values of serum hepatitis C virus RNA by branched DNA amplification assay in 52 consecutive patients with chronic hepatitis C immediately before high-dose treatment with interferon-alpha. Thirty-four out of 52 patients had >106.3 ([ap]2 x 106) equivalents/ml of viral genomes. Only three (8.8%) out of these 34 were long-term responders, while 16 (88.9%) out of 18 patients with 6.3 equivalents/ml of viral genomes were long-term responders (P 6.3 equivalents/ml and 10 (90.9%) out of these 11 had long-term response, while only one (3.2%) out of 31 patients with >106.3 equivalents/ml was a long-term responder (P < 0.001). By determining HCV-RNA levels in serum, we could thus predict long-term responsiveness to high-dose interferon therapy in 47 (90.4%) out of 52 chronic hepatitis C patients, and 40 (95.2%) out of 42 patients with genotype II hepatitis C virus. Our results indicate that quantitative measurement of hepatitis C viral genomes in pretreatment serum by the simple branched DNA amplification assay is an excellent method for predicting long-term responsiveness to interferon treatment.

http://www.sciencedirect.com/science/article/B6T7D-4F6DWMP-3C/2/cc0c722792d6cf3729d77800932971fe

The effect of interferon on hepatitis C virus RNA levels in the liver was studied by polymerase chain reaction in chronic hepatitis C patients who were enrolled into a pilot study with short-term interferon treatment. Among 17 patients treated with interferon [alpha] or [beta] (168-560 M.U. in total), eight were 'long-term responders' as defined by sustained normal serum alanine aminotransferase levels for more than 12 months, while the other nine were 'nonresponders' with abnormal alanine aminotransferase levels. In eight 'long-term responders', both the plus and minus strands (replicative intermediate) of hepatitis C virus RNA could not be detected in the liver after interferon treatment whereas eight of nine 'non-responders' retained the minus strand or both strands of hepatitis C virus RNA in the liver. Serum hepatitis C virus RNA was no longer detected in seven of nine 'non-responders' or in all 'long-term responders' at the end of treatment, but it reappeared in 'non-responders' with the elevation of serum alanine aminotransferase levels, which excluded serum hepatitis C virus RNA as a prognostic marker for sustained alanine aminotransferase normalization after interferon treatment. Our results indicate that the disappearance of hepatitis C virus RNA including the minus strand RNA from the liver is a predictive marker for good prognosis in chronic hepatitis C patients after interferon treatment. It is of great use to determine the levels of hepatitis C virus RNA in the liver to know the prognosis of interferon-treated patients who maintain sustained normal serum alanine aminotransferase levels and undetectable serum hepatitis C virus RNA after interferon treatment.

**International Immunopharmacology** (10)


http://www.sciencedirect.com/science/article/B6W7N-44F6DJV-7/2/5c7706d087732867371db915883d0256

An in vitro model of multi-step activation, in which cells of macrophage lineage are driven sequentially through inflammatory, primed, and fully activated states, was employed to assess for cannabinoid receptor expression. Murine and rat peritoneal macrophages, murine RAW264.7 and P388D1 macrophage-like cells, and neonatal rat brain cortex microglia expressed the cannabinoid receptor type 2 (CB2) differentially in relation to cell activation. The CB2 was undetectable in resident peritoneal macrophages, present at high levels in thioglycollate-elicited inflammatory and interferon gamma (IFN[gamma])-primed peritoneal macrophages, and detected at significantly diminished levels in bacterial lipopolysaccharide (LPS)-activated peritoneal macrophages. A comparable pattern of differential expression of the CB2 was noted for murine macrophage-like cells and neonatal rat brain cortex microglia. The cannabinoid receptor type 1 (CB1) was not detected in peritoneal macrophages or murine macrophage-like cells regardless of cell activation state but was present in neonatal rat microglia at low levels. These results indicate
that levels of the CB2 in cells of macrophage lineage undergo major modulatory changes in relation to cell activation. Furthermore, since inflammatory and primed macrophages express the highest levels of CB2, the functional activities of macrophages when in these respective states of activation may be the most sensitive to the action of cannabinoids.


Herbal medicines are increasingly being utilized to treat a wide variety of disease processes. Aqueous extract from the root of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK), is reported to have antitumor and immunomodulatory activities; however, the mechanism underlying its therapeutic effect is not known. In the present study we examined the effects of CK on the release of nitric oxide (NO) and tumor necrosis factor-[alpha] (TNF-[alpha]), and on the gene expression of iNOS and TNF-[alpha] in mouse macrophages. CK elicited a dose-dependent increase in NO and TNF-[alpha] production in cultured macrophages. CK significantly affected secretion at concentrations of more than 5 [mu]g/ml, and its maximum effect was at concentration of 100 [mu]g/ml. Reverse transcription polymerase chain reaction showed that increases in NO and TNF-[alpha] secretion were due to an increase in inducible NO synthase mRNA and TNF-[alpha] mRNA, respectively. Transient expression assays with NF-[kappa]B binding sites linked to the luciferase gene revealed that CK-induced increase of inducible NO synthase mRNA and TNF-[alpha] mRNA were mediated by the NF-[kappa]B transcription factor complex. These results demonstrate that CK stimulates NO and TNF-[alpha] release and is able to upregulate iNOS and TNF-[alpha] expression through NF-[kappa]B transactivation and this may be a mechanism whereby this herbal medicine elicits its therapeutic effects.


RNA oligonucleotides termed External Guide Sequence (EGS) and RNAi have been described that target specific gene expression by site-specific cleavage of mRNA. EGS serve as an RNA catalyst or ribozyme by directing bound mRNA to the ubiquitous cellular enzyme RNAse P. We describe an EGS targeting human interleukin (IL)-4 receptor [alpha] mRNA, an important cytokine receptor in the pathogenesis of asthma and allergic disease expressed in pulmonary tissues. This EGS was designed to explore pulmonary delivery of catalytic RNA oligonucleotides as a novel therapy in asthma and other atopic diseases. Inhaled DNA oligonucleotides termed Respirable Antisense Oligonucleotide Sequences (RASONS) are selectively internalized in lung tissues in a complex with endogenous lipid surfactants present in normal lung and can alter pulmonary gene expression. Potential applications of inhaled RNA oligonucleotides in therapy of pulmonary and related systemic diseases are discussed.

Esser, C., V. Temchura, et al. (2004). "Signaling via the AHR leads to enhanced usage of CD44v10 by murine fetal thymic emigrants: possible role for CD44 in emigration." International...
Signaling via the endogenous arylhydrocarbon receptor (AHR) affects proliferation, differentiation, function and gene expression of thymocytes. In the present study, we show that treatment of mouse fetal thymus lobes in organ culture (FTOC) with AHR ligands results in (a) a drastic decrease in the emigration of thymocytes in terms of numbers and types of cells, and (b) preferential emigration of CD4-CD8- (DN) cells expressing CD44v7- and CD44v10-containing isoforms on the cell surface. Moreover, a higher level of transcripts of various other CD44 variant isoforms (CD44v) could be detected by RT-PCR in emigrants from fetal thymi exposed to either AHR-agonist during culture. Expression of CD44v9-10-containing isoforms could be exclusively detected in DN thymic emigrants. Thus, signaling via AHR by ligands alters CD44v expression patterns in a thymocyte subpopulation. Furthermore, emigration could be decreased by the addition of anti-panCD44 antibodies to TCDD-treated FTOCs, suggesting a role for CD44 in emigration.

BackgroundProstaglandin E2 (PGE2) is known to modulate immune responses and is widely viewed as a general immunosuppressant. There have been recognized four receptors for PGE2 (EP1-EP4 receptor) so far, and EP2 and EP4 receptors are mainly involved in the immunosuppressive effect of PGE2 in vitro. In the present study we examined the in vivo immunosuppressive effects of selective EP receptor agonists using a high-responder rat skin transplantation model. Materials and methods Skin allografts from ACI donors were transplanted onto LEW recipients. Agents were injected everyday between day 0 and day 5 after skin transplantation at the dose of 300 [mu]g/kg subcutaneously. Survival of the skin allograft, histological changes and changes of the intragraft cytokine expressions were analyzed using the reverse transcription polymerase chain reaction (RT-PCR). We also assessed the mixed lymphocyte reaction (MLR) assay using splenocytes. Results PGE2 significantly prolonged allograft survival (18.8 [plus-or-minus sign] 1.5 days) compared with untreated control (14.8 [plus-or-minus sign] 0.8 days). EP2R + EP3R + EP4R agonists also prolonged allograft survival (18.0 [plus-or-minus sign] 1.0 days) although EP3R agonist or EP2R + EP4R agonists alone failed (15.5 [plus-or-minus sign] 0.7, 15.4 [plus-or-minus sign] 1.3 days, respectively). RT-PCR analysis in the skin grafts demonstrated IL-10 up-regulation and IFN-[gamma] down-regulation in all groups except untreated control and EP2R agonist-treated groups. MLR was significantly reduced in groups of EP2R + EP4R agonists, EP2R + EP3R + EP4R agonists and PGE2, compared with untreated control. Conclusions The effect of PGE2 to prolong the survival of skin transplant requires the action of a combination of three receptors, i.e., EP2 + EP3 + EP4.
Many polysaccharides isolated from plants are considered to be biological response modifiers and have been shown to enhance various immune responses in vivo and in vitro. Here, we demonstrate that polysaccharide isolated from the radix of Platycodon grandiflorum (PG) has a unique mode of immunostimulation with regard to its cell-type specificity. PG was found to markedly increase polyclonal IgM antibody production and the proliferation of B cells, and to activate iNOS transcription and NO production in macrophages. Moreover, the intraperitoneal administration of PG in mice resulted in increased IgM antibody production in B cells, which were immunized by using T-dependent antigen sheep red blood cells (sRBCs). However, PG did not affect the proliferation of T cells, the IL-2 expression of Th1 cells, or the IL-4 expression of Th2 cells. Although PG and lipopolysaccharide (LPS) had a similar mode of action in B cells and macrophages, they were differentiated by the fact that PG-induced cellular activation was not inhibited by polymyxin B, a specific inhibitor of LPS. Anti-CD19 or anti-CD79b antibody blocked B cell proliferation and anti-CD14 or anti-CD11b antibody decreased macrophage NO production, indicating the possible cellular binding sites of PG. Our results demonstrate that PG is a specific activator of B cells and macrophages but not of T cells, and suggest that PG is quite distinct from other well-known immunostimulants, such as lentinan and schizophyllan, which mainly act upon macrophages and T cells.


http://www.sciencedirect.com/science/article/B6W7N-48342TC-1/2/cb01711e3e2dbe29e676251ba0d851ed

We investigated the immunomodulatory activity of polysaccharide isolated from the root of Acanthopanax koreanum (AK) at the cellular level. AK directly increased B cell proliferation and antibody production, but did not affect the expression of IL-2, IFN-[gamma] or IL-4 by T cells, or T cell proliferation in vitro. Since AK cannot penetrate cells due to its large molecular mass, B cell activation may be caused by the surface binding of AK to B cell-specific receptors. The role of TLR4 as an AK receptor was shown by the fact that AK activity in B cells from C3H/HeJ mice, which are known to have a defective Toll-like receptor (TLR)-4, was found to be reduced compared with that in control cells from C3H/HeN mice. AK activity was also reduced by antibodies blocking TLR2, TLR4, CD19 or CD79b, but not by an antibody blocking CD38, which suggests AK receptor profiling in B cells. Two main differences between AK and lipopolysaccharide (LPS) were observed. First, LPS activity was inhibited by antibodies to either TLR2 or TLR4, but not by antibodies to CD19, CD79b or CD38. Another was that LPS-induced B cell proliferation was inhibited by polymyxin B (PMB), a specific inhibitor of LPS, whereas AK activity was not affected. Taken together, our results demonstrate that AK directly activates B cells, but not T cells, and suggest that AK has a broader receptor profile than LPS in B cells.


http://www.sciencedirect.com/science/article/B6W7N-48NC18X-1/2/2b271d513223ab76590f458722074008

We investigated the mechanism of the immunomodulatory action of polysaccharide (ASP) isolated from a cell culture of Acanthopanax senticosus. ASP was found to directly increase the proliferation and differentiation of B cells, and the cytokine production of macrophage, but not the proliferation and cytokine production of T cells. Since ASP cannot penetrate the cell membrane due to its large molecular mass, such cellular activation may be caused by the surface binding of
ASP to receptors expressed on B cells and macrophages. The possibility that TLRs, which are known to be involved in immune-related responses, may be the receptor(s) of ASP was investigated. The immunomodulating activities of ASP on the B cells and macrophages of C3H/HeJ mice, expressing a defective toll-like receptor (TLR)-4, were decreased versus the corresponding cells from C3H/HeN mice. In addition, the activities of ASP on B cells and macrophages were significantly reduced by treating the cells with antibodies to TLR4 and TLR2 prior to ASP, suggesting that both of them are the possible receptors of ASP. The ligation of TLRs induced by ASP was able to activate mitogen-activated protein kinases (MAPKs), such as Erk1/2, p38 and JNK, and the transcription factor NF-κB. Although ASP was shown to activate the TLR signaling cascades in the same manner as lipopolysaccharide (LPS), these two could be differentiated by the finding that polymyxin B (PMB), a specific inhibitor of LPS, did not significantly affect the activities of ASP on B cells and macrophages. Taken together, our results demonstrate that ASP, isolated from a cell culture of A. senticosus, activates B cells and macrophages by interacting with TLRs and leading to the subsequent activation of mitogen-activated protein kinases and NF-κB.


http://www.sciencedirect.com/science/article/B6W7N-477GGBF-1/2/a22126c7c0a45992f589c6d51973a575

Interleukin-12 (IL-12) is a heterodimeric cytokine that enhances immune responses to bacterial, parasitic, and viral pathogens, and leads to tumor regression in animal models. For this reason, the use of IL-12 as a vaccine adjuvant and as a therapeutic agent for the treatment of cancer is being investigated. Unfortunately, the extreme toxicity of this molecule observed during clinical trials has limited its use. This toxicity correlates with increased IFN-γ expression, decreased glucose levels, and altered histological responses in the spleen and duodenum. In this study, we show that intranasal (i.n.) delivery of IL-12 is a less toxic route of inoculation compared to the commonly employed subcutaneous route. When delivered i.n., IL-12 induces less systemic IFN-γ production and fewer pathological tissue changes, yet is efficacious, as indicated by enhanced CD3+ T cell activation and increased production of Th1-associated immunoglobulins (i.e., serum IgG2a). Thus, IL-12 can be delivered safely and effectively by the i.n. route, a finding which may allow IL-12 to fulfill its clinical potential.


http://www.sciencedirect.com/science/article/B6W7N-4619CVP-2/2/ce1ffbc9b72ee4d3f2754b6db61912f3

Herein we report mechanisms whereby Flt3 ligand (FL) augments steady state T cell activity in addition to the expansion of dendritic cells (DCs). We demonstrate that in vivo administration of FL increases the frequency and absolute number of effector/memory T cells and preferentially expands T cells that express a type-1 cytokine phenotype. In addition, FL enhances T cell proliferative responses to Concanavalin A that directly correlated with increased frequencies in effector/memory T cells and expansion of lymphoid-derived (type 1) DCs (DC1s). Together, these data demonstrate that mechanisms of FL-induced T cell regulation include not only the expansion of DC subsets, but also the preferential expansion of type 1-effector/memory T cell populations, and suggest multiple mechanisms of action for FL as a vaccine adjuvant and as a therapeutic modality.

http://www.sciencedirect.com/science/article/B6T7F-4961NGV-1/2/4bc2cd1918c679086a7fe5dbf9d58f71

Toxoplasma gondii is associated with morbidity and mortality in a variety of marine mammals, including fatal meningoencephalitis in the southern sea otter (Enhydra lutris nereis). The source(s) of T. gondii infection and routes of transmission in the marine environment are unknown. We hypothesise that filter-feeding marine bivalve shellfish serve as paratenic hosts by assimilation and concentration of infective T. gondii oocysts and their subsequent predation by southern sea otters is a source of infection for these animals. We developed a TaqMan PCR assay for detection of T. gondii ssrRNA and evaluated its usefulness for the detection of T. gondii in experimentally exposed mussels (Mytilus galloprovincialis) under laboratory conditions. Toxoplasma gondii-specific ssrRNA was detected in mussels as long as 21 days post-exposure to T. gondii oocysts. Parasite ssrRNA was most often detected in digestive gland homogenate (31 of 35, i.e. 89%) compared with haemolymph or gill homogenates. Parasite infectivity was confirmed using a mouse bioassay. Infections were detected in mice inoculated with any one of the mussel sample preparations (haemolymph, gill, or digestive gland), but only digestive gland samples remained bioassay-positive for at least 3 days post-exposure. For each time point, the total proportion of mice inoculated with each of the different tissues from T. gondii-exposed mussels was similar to the proportion of exposed mussels from the same treatment groups that were positive via TaqMan PCR. The TaqMan PCR assay described here is now being tested in field sampling of free-living invertebrate prey species from high-risk coastal locations where T. gondii infections are prevalent in southern sea otters.


http://www.sciencedirect.com/science/article/B6T7F-45RFM7B-5/2/04158c2ab3ac5dac750db5c8531b9c44

DNA was extracted from 71 meat samples obtained from UK retail outlets. All of these DNA preparations gave the expected polymerase chain reaction products when amplified with primers specific for the species from which the meat originated. A second polymerase chain reaction analysis, using primers specific for the Toxoplasma gondii SAG2 locus, revealed the presence of this parasite in 27 of the meat samples. Restriction analysis and DNA sequencing showed that 21 of the contaminated meats contained parasites genotyped as type I at the SAG2 locus, whilst six of the samples contained parasites of both types I and II. Toxoplasma- positive samples were subjected to further polymerase chain reaction analysis to determine whether any carried an allele of the dihydropteroate synthase gene that has recently been shown to be causally associated with sulfonamide resistance in T. gondii. In all cases, this analysis confirmed that parasites were present in the samples and, additionally, revealed that none of them carried the
drug-resistant form of dihydropteroate synthase. These results suggest that a significant proportion of meats commercially available in the UK are contaminated with T. gondii. Although none of the parasites detected in this study carried the sulfonamide-resistance mutation, a simplified procedure for monitoring this situation merits development.


http://www.sciencedirect.com/science/article/B6T7F-4447KS1-C/2/769a78f65756bb6318627c7be539ed869

We describe a highly sensitive real-time PCR to detect and measure the development of the liver-stages of malaria parasites in mice infected with sporozoites ranging in number from 25 to more than 164,000, using the same reaction conditions. Furthermore, this assay detects and measures parasite loads in the livers of mice exposed to the bite of a single malaria-infected Anopheles mosquito. This unique method should greatly facilitate studies aimed at evaluating very precisely the efficacy of anti-malarial experimental drug treatments and vaccination regimens in conditions of infection resembling those found in the field.


http://www.sciencedirect.com/science/article/B6T7F-3WF7DY2-4/2/e199df576315ec9f38d3b00d1c31081

The larvae of the fly Lucilia cuprina excrete or secrete a chymotrypsin (LCTb) onto the skin of sheep to facilitate the establishment of the larval infestation. A combination of immunoblotting and RT-PCR approaches has established that this protease is also a gut digestive protease. LCTb is synthesized primarily in the cardia, a small highly specialized organ located at the anterior end of the midgut and by midgut cells. There is also some expression by the hindgut but no expression by salivary glands. Excretion of LCTb with waste products or regurgitation of the gut contents of the larvae may explain how this protease is transferred from the larval gut onto ovine skin. LCTb is first expressed in eggs and constitutively expressed throughout each larval instar, but is not expressed in pupae or adult flies. It is concluded that LCTb could be involved in the establishment of larvae on sheep skin as well as acting as a general gut digestive enzyme.


http://www.sciencedirect.com/science/article/B6T7F-4FH5GV9-2/2/12e8d0f05ff570d5204b0bcb2989e6f1

A study was undertaken to compare the performance of five different molecular methods (available in four different laboratories) for the identification of Cryptosporidium parvum and Cryptosporidium hominis and the detection of genetic variation within each of these species. The same panel of oocyst DNA samples derived from faeces (n=54; coded blindly) was sent for analysis by: (i) DNA sequence analysis of a fragment of the HSP70 gene; (ii) DNA sequence
analysis and the ssrRNA gene in laboratory 1; (iii) single-strand conformation polymorphism analysis of part of the ssrRNA; (iv) SSCP analysis of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA region in laboratory 2; (v) 60 kDa glycoprotein (gp60) gene sequencing with prior species determination using PCR with restriction fragment length polymorphism analysis of the ssrRNA gene in laboratory 3; and (vi) multilocus genotyping at three microsatellite markers in laboratory 4. For detecting variation within C. parvum and C. hominis, SSCP analysis of ITS-2 was considered to have superior utility and determined 'subgenotypes' in samples containing DNA from both species. SSCP was also most cost effective in terms of time, cost and consumables. Sequence analysis of gp60 and microsatellite markers ML1, ML2 and 'gp15' provided good comparators for the SSCP of ITS-2. However, applicability of these methods to other Cryptosporidium species or genotypes and to environmental samples needs to be evaluated. This trial provided, for the first time, a direct comparison of multiple methods for the genetic characterisation of C. parvum and C. hominis samples. A protocol has been established for the international distribution of samples for the characterisation of Cryptosporidium. This can be applied in further evaluation of molecular methods by investigation of a larger number of unrelated samples to establish sensitivity, typability, reproducibility and discriminatory power based on internationally accepted methods for evaluation of microbial typing schemes.


http://www.sciencedirect.com/science/article/B6T7F-42XB4F7-C/2/bc45a0d3915e84b1e2cdff41e53cea7c

A diagnostic ELISA with recombinant Fasciola hepatica cathepsin L-like protease as antigen was developed to detect antibodies against F. hepatica in sheep and cattle. The recombinant cathepsin L-like protease was generated by functional expression of the cDNA from adult stage F. hepatica flukes in Saccharomyces cerevisiae. Specificity and sensitivity of the cathepsin L enzyme-linked immunosorbent assay (ELISA) was assessed using sera from sheep and calves experimentally or naturally mono-infected with F. hepatica and six-seven other parasites. The sensitivity of the cathepsin L ELISA for sheep and cattle sera was 99.1 and 100%, respectively. In the experimental setting with established mono-infections, the specificity of the cathepsin L ELISA was 98.5% for cattle sera and 96.5% for sheep sera. In experimentally infected cattle and sheep, the first detection of F. hepatica-specific antibodies appeared first between 5 and 7 weeks post-infection, but depended on the infectious dose of F. hepatica. In ELISA the detection preceded first detection of the infection based on egg counts and remained detectable till at least 23 weeks after a primary F. hepatica infection. Detection of Fasciola gigantica infections was similar to detection of F. hepatica. The first detection occurred at week 5 and signals persisted for at least 20 weeks. All sera from naturally F. hepatica infected sheep were seropositive in the cathepsin L-like ELISA. The relevance of this ELISA format was also evaluated using sera from naturally infected cattle in the Netherlands, Ecuador and Vietnam and compared with results from egg-counts. For the latter two endemic areas with mixed parasitic infections the 'apparent' sensitivity of the cathepsin L ELISA was calculated for all serum samples together to be 90.2%. The 'apparent' specificity under these conditions was calculated to be 75.3%. In cattle, the cathepsin L ELISA was superior to the concurrently evaluated peptide ELISA format using a single epitope as the antigen both in controlled natural infections as well as in infections in endemic areas. The present ELISA-format contributes a relatively sensitive and reliable tool for the early serodiagnosis of bovine and ovine fasciolosis.

The polymerase chain reaction (PCR) has been used to amplify a 0.52 kb segment of Giardia intestinalis DNA, using primers specific for nucleotide sequences conserved within two genes (tspl1 and tsa417) that encode homologous, cysteine-rich trophozoite surface proteins. Using products amplified from axenic isolates belonging to genetic groups I and II (defined on the basis of allozyme electrophoresis data), restriction endonuclease analysis revealed both tsp11-like and tsa417-like fragments within all samples. The study also identified among the amplification products of group II organisms an additional fragment, containing a novel PstI site, that is not detected in the reaction products of group I isolates. The recovery of three distinct PCR products from each group II isolate was verified by cloning the fragments into the plasmid vector pGEM-7. Fragments containing the new PstI site possess the ClaI site common to both tsp11 and tsa417-like fragments, but they lack the HindIII site which characterizes tsp11-like fragments and also lack the PstI and KpnI sites which characterize tsa417-like fragments. Spot-blot analyses using cloned fragments of all three types as probes showed strong homologous hybridization but weak heterologous hybridization, indicating that each type diners substantially in nucleotide sequence from the others. Because the samples of Giardia DNA used in the PCR were purified from cultures that had been established from single trophozoites, the data indicate that individual trophozoites belonging to genetic group II possess three homologous genes defined by these related fragments. The presence of a PstI site in the amplified segment of the newly-discovered third gene of group II organisms provides a simple diagnostic means of differentiating group I and II isolates.

The parasitic nematode, Brugia malayi, causes lymphatic filariasis in humans, which in severe cases leads to the condition known as elephantiasis. The parasite contains an endosymbiotic [alpha]-proteobacterium of the genus Wolbachia that is required for normal worm development and fecundity and is also implicated in the pathology associated with infections by these filarial nematodes. Bacterial artificial chromosome libraries were constructed from B. malayi DNA and provide over 11-fold coverage of the nematode genome. Wolbachia genomic fragments were simultaneously cloned into the libraries giving over 5-fold coverage of the 1.1 Mb bacterial genome. A physical framework for the Wolbachia genome was developed by construction of a plasmid library enriched for Wolbachia DNA as a source of sequences to hybridise to high-density bacterial artificial chromosome colony filters. Bacterial artificial chromosome end sequencing provided additional Wolbachia probe sequences to facilitate assembly of a contig that spanned the entire genome. Wolbachia sequences provided a marker approximately every 10 kb. Four rare-cutting restriction endonucleases were used to restriction map the genome to a resolution of approximately 60 kb and demonstrate concordance between the bacterial artificial chromosome clones and native Wolbachia genomic DNA. Comparison of Wolbachia sequences to public databases using BLAST algorithms under stringent conditions allowed confident prediction of 69 Wolbachia peptide functions and two rRNA genes. Comparison to closely related complete genomes revealed that while most sequences had orthologs in the genome of the Wolbachia endosymbiont from Drosophila melanogaster, there was no evidence for long-range synteny. Rather, there were a few cases of short-range conservation of gene order extending over regions of less than 10 kb. The molecular scaffold produced for the genome of the Wolbachia from B. malayi forms the basis of a genomic sequencing effort for this bacterium, circumventing the difficult challenge of purifying sufficient endosymbiont DNA from a tropical...
parasite for a whole genome shotgun sequencing strategy.


http://www.sciencedirect.com/science/article/B6T7F-478R723-9/2/fd8afa81941918772437340e711e311

Gale K. R., Dimmock C. M., Gartside M. & Leatch G. 1996. Anaplasma marginale: detection of carrier cattle by PCR-ELISA. International Journal for Parasitology 26:1103-1109. A highly sensitive and specific polymerase chain reaction (PCR) based assay for the detection of the minute levels of Anaplasma marginale present in the blood of long-term carrier cattle was developed. A simple lysis method was used to remove most of the haemoglobin from the blood to facilitate direct input of samples into the PCR reactions without prior purification of the DNA. PCR product was detected by enzyme-linked immunosorbent assay (ELISA) to simplify the processing of large numbers of samples. The sensitivity limit of the PCR-ELISA was 0.00015% parasitaemia (24 infected erythrocytes per microlitre of blood). No cross-reactivity of the assay was observed when A. marginale-negative blood infected with Babesia bovis or Theileria orientalis was tested. The PCR-ELISA was shown to be 92% efficient in the detection of long-term A. marginale carrier cattle. No false-positive results were obtained. These results compared favourably with 2 serological assays for detection of A. marginale carrier cattle (card agglutination test and ELISA) which were applied to the same experimental animals.


http://www.sciencedirect.com/science/article/B6T7F-4BMJ9GJ-2/2/3793794b640a6be2cb9b5a2e64e427a9

Cathepsin L (CL)-like proteases are important candidate vaccine antigens for protection against helminth infections. We previously identified an immunogenic 32 kDa protein specifically present in newly excysted juveniles (NEJs) of Fasciola hepatica. Here we show by N-terminal protein sequencing that this protein represents a CL-like protease still containing the propeptide. Two cDNAs encoding this CL were subsequently isolated from NEJs by RT-PCR. The predicted amino acid sequences of these cDNAs showed approximately 70% sequence homology to both CL1 and CL2 sequences isolated from adult stage F. hepatica and are, therefore, referred to as CL3. The CL3 clones encoded asparagine at position P1 of the propeptide cleavage site, suggesting a dependence on asparaginyl endopeptidases for maturation. Recombinant expression of a CL3 cDNA in Saccharomyces cerevisiae resulted in secretion of the proenzyme form. The propeptide of CL-like proteins was predicted to contain important B-cell epitopes. To determine the contribution of the propeptide to protective immunity, rats were vaccinated with Keyhole Limpet Haemocyanin-conjugated synthetic peptides encoding these putative B-cell epitopes derived from the CL1 or CL3 sequence. A subsequent challenge infection resulted in a significant (PF. hepatica infection.

Here, we report evaluation of five oligoprobes designed from intergenic spacer (IGS) region sequences for identification of cyathostomin species. Oligoprobes were designed for identification of Cylicocyclus ashworthi, Cylicocyclus nassatus, Cylicostephanus longibursatus, Cylicostephanus goldi and a fifth probe designed to identify all members of this tribe. PCR amplification of IGS DNA from 16 cyathostomin species allowed sequence comparison and identification of four putative species-specific probes. Southern blotting of amplified products from 16 species showed that all probes were species-specific. The fifth probe recognised all 16 cyathostomin species but did not bind to members of the genus Strongylus. Furthermore, these probes were used to identify individual infective L3, eggs and L4 indicating that they will be invaluable to furthering the study of the epidemiology and pathogenesis of these important equine nematodes.


Total DNA was isolated from adult lungworms of the genus Dictyocaulus, collected from cattle, moose (Alces alces) and roe deer (Capreolus capreolus) in Sweden. The second ribosomal internal transcribed spacer was amplified with PCR, and DNA sequences were determined from nine individual worms that all came from different hosts in order to avoid analysis of siblings. The sequence data obtained were aligned and compared with similar data derived from German lungworm isolates from cattle and fallow deer (Cervus dama). These analyses clearly showed that specimens of the cattle lungworm, Dictyocaulus viviparus, were almost identical irrespective of their geographical origin. However, when the second internal transcribed spacer sequence of D. viviparus was compared with that of lungworms from moose and roe deer, major differences were noticed. Although lungworms collected from these cervids had identical second internal transcribed spacer sequences, they proved to be genetically different from Dictyocaulus eckerti of German fallow deer, displaying a 66.5% similarity. In an evolutionary tree, inferred by maximum likelihood analysis, the Dictyocaulus species from cattle and wild cervids clustered as compared with Dictyocaulus filaria from sheep. The study has thus demonstrated that A. alces and C. capreolus in Sweden are parasitised with a Dictyocaulus species that is different from D. viviparus and D. eckerti, indicating that we are dealing with a new species in moose and roe deer.


EtCRK2, a cyclin-dependent kinase from the coccidian parasite, Eimeria tenella is closely related to eukaryotic cyclin-dependent kinases that regulate progression of the cell cycle and to several cyclin-dependent kinases identified in the Apicomplexa. Northern blot analyses revealed that EtCRK2 is transcribed during both asexual (first-generation schizogony) and sexual (oocyst sporulation) replicative phases of the parasite life cycle. In addition, it appears to be
transcriptionally regulated during meiosis. Recombinant EtCRK2 produced in Escherichia coli has kinase activity which is significantly stimulated by the addition of vertebrate cyclin A. This cyclin-dependent kinase may play a significant role in regulating critical cell cycle events during both asexual proliferation and sexual development of the parasite.


http://www.sciencedirect.com/science/article/B6T7F-3YRVKCC-7/2/65f1d53d0276d04351610b1c7ca1b9b8

To facilitate studies of vaccines and antimicrobial agents effective against Toxoplasma gondii infection, an assay system was developed to semi-quantitate parasitaemia using PCR amplification of T. gondii DNA obtained from the blood of mice infected with the parasite. A competitive internal standard DNA fragment of the B1 gene of T. gondii was generated and used in PCR so that the amplified product could be semi-quantitated and false negative results could be avoided. The PCR assay system was used to analyse the levels of parasitaemia in immunised and antimicrobial agent treated mice at various times after infection with T. gondii. The results of these studies indicate that this highly sensitive detection method is a rapid and reliable procedure that can be employed to assess the abilities of vaccines or antimicrobial agents to provide protection early following T. gondii infection.


http://www.sciencedirect.com/science/article/B6T7F-4DXTJJT-1/2/ac446912b091bf6f7b59af1d2d730ff5

Human giardiasis, caused by the intestinal flagellate Giardia duodenalis, is considered a zoonotic infection, although the role of animals in the transmission to humans is still unclear. Molecular characterisation of cysts of human and animal origin represents an objective means to validate or reject this hypothesis. In the present work, cysts were collected in Italy from humans (n=37) and animals (dogs, one cat and calves, n=46), and were characterised by PCR amplification and sequencing of the [beta]-giardin gene. As expected, only Assemblages A and B were identified among human isolates. The host-specific Assemblages C and D were found in the majority of dog isolates; however, 6 dog isolates were typed as Assemblage A. The cat-specific Assemblage F has been identified in the single feline isolate available. Among calf isolates, most were typed as Assemblages A (n=12) and B (n=5), whereas the host-specific Assemblage E was rarely found (n=3). Sequence heterogeneity in the [beta]-giardin gene allowed a number of subgenotypes to be identified within Assemblage A (8 subgenotypes), B (6 subgenotypes), D (2 subgenotypes), and E (3 subgenotypes). Five of these subgenotypes, namely A1, A2, A3, A4 and B3, were found to be associated with infections of humans, of dogs and of calves; these data, therefore, supported the role of these animals as a source of infection for humans.

Fifty fresh isolates of Trypanosoma cruzi from Triatoma dimidiata vectors and 31 from patients with Chagas disease were analysed for DNA polymorphisms within the 432-bp core region of the cruzipain gene which encodes the active site of cathepsin L-like cystein proteinase. The cruzipain gene showed signs of polymorphism consisting of four different DNA sequences in Central and South American isolates of T. cruzi. The PCR fragments of Guatemalan isolates could be divided into three groups, Groups 1, 2 and 3, based on different patterns of single-stranded DNA conformation polymorphism. All of the strains isolated from Brazil, Chile, and Paraguay, except for the CL strain, showed a Group 4 pattern. Two to four isolates from each group were analysed by cloning and sequencing. A silent mutation occurred between Groups 1 and 2, and five nucleotides and two aa substitutions were detected between Groups 1 and 3. The DNA sequence of Group 4 contained five nucleotides and one aa substitution from Group 1. All of the DNA sequences corresponded well with the single-stranded DNA conformation polymorphism. The Group 1 isolates, the majority in the Guatemalan population (70/81, 86.4%), were isolated from both triatomines and humans, but Group 3 were isolated only from humans. Moreover, the Group 2 isolates were detected only in triatomine vectors (9/50; 18%), but never in humans (0/32, P<0.05) suggesting that this group has an independent life-cycle in sylvatic animals and is maintained by reservoir hosts other than humans.


Partial nuclear 28S ribosomal RNA and mitochondrial cytochrome c oxidase subunit I (COI) gene sequences (953 and 385 nucleotides, respectively) of one fish monogenean (outgroup) and six polystome monogeneans (four Polystomoides spp. from the oral cavities and urinary bladders of freshwater turtles in Australia and Malaya, two Neopolystoma spp. from the urinary bladder and conjunctival sac of a freshwater turtle in Australia) were used to examine the question of whether congeneric species infecting different sites in the same host species have speciated in that host by adapting to different sites, or whether species infecting a particular site in one host have given rise to species infecting the same site in different hosts. Results show unequivocally that congeneric species infecting the same site, even of host species belonging to different suborders and occurring on different continents, are more closely related than congeneric species infecting different sites of the same host species. This is interpreted as meaning that speciation has not occurred in one host. Morphological evolution of polystomes has been very slow: few differences between species and even genera have evolved over a period of at least 150 Myr, and this is matched by low substitution rates of nucleotides, and the ambiguous position of species of different genera, depending on whether COI or 28S rDNA sequences are used.

a variety of platyhelminths, places the enigmatic Udonella caligorum firmly as a monopisthocotylean monogenean. Both maximum parsimony and a modified distance measure, operating under a maximum likelihood model, gave identical solutions for each data set. These data further support morphological evidence from ultrastructural studies indicating the neodermatan affinities of Udonella, namely shared features in sensory receptors, surface tegument, sperm structure and spermiogenesis. The molecular data reject the class Udonellidea and the placement of udonellids as sister-group to the Neodermata. As shown previously with molecular data, the monogeneans appear as a paraphyletic assemblage comprising strongly monophyletic Monopisthocotylea and Polyopisthocotylea. Their relationships with the trematodes and cestodes are not resolved with 28S rDNA or 18S rDNA alone.


http://www.sciencedirect.com/science/article/B6T7F-3W1RGB1-1D/2/6aa09e0dd72c08f89012724348195d38

Giardia lamblia (also Giardia duodenalis, Giardia intestinalis) isolates have been variably divided into two or three genotypes by different investigators. We have compared the triose phosphate isomerase sequences of the three genotypes (Groups 1, 2, and 3) described by Nash and shown that Groups 1 and 2 are similar, while Group 3 is markedly different from Groups 1 and 2, indicating that Group 1/2 and Group 3 correspond to the two major genotypes identified by other investigators. We have also analysed three Chinese isolates and showed that two fit into Group 3, while the third contained a mixture of Groups 1 and 3 isolates. These results confirm the relatedness of G. lamblia isolates from throughout the world, and established the feasibility of using DNA amplification and sequence analysis for detecting mixed isolates.


http://www.sciencedirect.com/science/article/B6T7F-476FF6X-154/2/9ff9371dda2e625023b1703fa1358269

African trypanosome species were identified using the Polymerase Chain Reaction (PCR) by targeting repetitive DNA for amplification. Using oligonucleotide primers designed to anneal specifically to the satellite DNA monomer of each species/subgroup, we were able to accurately identify Trypanosoma simiae, three subgroups of T.congolense, T.brucei and T.vivax. The assay was sensitive and specific, detecting one trypanosome unequivocally and showing no reaction with non-target trypanosome DNA or a huge excess of host DNA. The assay was used to identify developmental stage trypanosomes in the tsetse fly. The use of radioisotopes was not necessary and mixed infections could be detected easily by incorporating more than one set of primers in a single reaction. The use of crude preparations of template made the process very rapid. The methodology should be suitable for large-scale epidemiological studies.


http://www.sciencedirect.com/science/article/B6T7F-3YF49VW-27/2/f83e029b52ec29f78a0d375a4b6ef3bd
Previous studies detected a single amino acid substitution (Ala196 to Gly196) between cDNA clones encoding a 32 kDa antigen (p32) of Theileria sergenti (Chitose stock) obtained from a persistently infected calf. In this study, 2 different recombinant baculoviruses (pAc/p32-Ala196 and pAc/p32-Gly196) were constructed for the expression of p32. Molecular masses of the polypeptides produced in Spodoptera frugiperda cells infected with the recombinant baculoviruses were the same as that of anheutic p32. pAc/p32-Ala196 produced additional polypeptides, with molecular masses higher than 32 kDa, which resulted from differential N-glycosylation as revealed by endo N-glycosidase treatment. The results indicate that a single amino acid substitution may lead to a conformational change in p32 which affected post-translational modification of recombinant products.


http://www.sciencedirect.com/science/article/B6T7F-42D811P-B/2/d7f6eb70615674a822328aba6c26ec

Dictyocaulus viviparus causes a serious lung disease of cattle. For over 30 years, a radiation-attenuated larval vaccine has been used with success; however, this vaccine has several disadvantages. A more stable vaccine against D. viviparus, capable of stimulating prolonged protective immunity, would be beneficial. Recent research has been directed at adult worm ES components that may be involved in parasite survival in the host. One component is the secreted enzyme, acetylcholinesterase (AChE), a target for circulating antibody in infected calves. Here, we describe a study where protection was investigated in calves immunised with either native adult ES products or a recombinant parasite AChE. These antigens were administered twice with Freund's incomplete adjuvant. Subsequently, all calves were challenged with 700 L3 and their worm burdens and immune responses compared with those in calves that received an anthelmintic-abbreviated infection and challenge control calves. Significant levels of protection were not obtained in the immunised groups but significant immunity was achieved in the calves that received the anthelmintic abbreviated infection. Antibody responses amongst the groups were different, with significantly higher IgG1 responses in the immune, infected group and in adult ES recipients. Significantly higher IgG2 responses were found in the latter group. Following challenge, the groups that received the abbreviated infection and the fusion protein produced specific antibody that bound the native enzyme. No differences were observed between groups in peripheral blood mononuclear cell responsiveness to either antigen. However, adult ES products appeared to have a mitogenic effect on these cells, whilst the fusion protein exhibited an inhibitory effect. These results suggest that in this form, AChE is not a potential vaccine candidate and that adult ES products, in contrast to previous experiments in guinea pigs, do not contain protective components.


http://www.sciencedirect.com/science/article/B6T7F-40PRGMX-1/2/71358285493327d604f340d5399edcd0

The tick Amblyomma triguttatum triguttatum has previously been reported from Western Australia, Queensland and New South Wales. A viable population of this species, including all developmental stages, has now been discovered on the southern end of Yorke Peninsula, South Australia. Species determination was carried out morphologically and using 18S and 16S rRNA. The data for 16S rRNA are the first published for this species. Amblyomma t. triguttatum is
significant through its involvement in the natural, Australian cycle of Coxiella burnetti, the
pathogen causing Q fever. The environment of Yorke Peninsula contains all of the components
required for a natural Q fever cycle and three cases of this disease have been reported from this
area since 1995. These findings reinforce the need to put in place effective mechanisms to
monitor parasite distributions at a time of large scale global change.

coccidia (Sporozoa)." International Journal for Parasitology 34(4): 501.

http://www.sciencedirect.com/science/article/B6T7F-4B71912-B/2/1711750abc739b647d44cc23b1feb1b8

There is no current comprehensive assessment of the molecular phylogeny of the coccidia, as all
recently published papers either deal with subsets of the taxa or sequence data, or provide non-
robust analyses. Here, we present a comprehensive and consistent phylogenetic analysis of the
available data for the small-subunit ribosomal RNA gene sequence, including a number of taxa
not previously studied, based on a Bayesian tree-building analysis and the covariotide model of
evolution. The assumptions of the analysis have been rigorously tested, and the benefits and
limitations highlighted. Our results provide support for a number of prior conclusions, including the
monophyly of the families Sarcocystidae (cyst-forming coccidia) and Eimeriidae (oocyst-forming
coccidia), but with bird-host Isospora species in the Eimeriidae and mammal-host species in the
Sarcocystidae. However, it is clear that a number of previously reported relationships are
dependent on the evolutionary model chosen, such as the placements of Goussia janae,
Lankesterella minimia and Caryospora bigenetica. Our results also confirm the monophyly of the
subfamilies Toxoplasmatinae and Sarcocystinae, but only some of the previously reported groups
within these subfamilies are supported by our analysis. Similarly, only some of the previously
reported groups within the Eimeriidae are supported by our analysis, and the genus Eimeria is
clearly paraphyletic. There are unambiguous patterns of host-parasite relationship within the
coccidia, as most of the well-supported groups have a consistent and restricted range of hosts,
with the exception of the Toxoplasmatinae. Furthermore, the previously reported groups for which
we found no support all have a diverse range of unrelated hosts, confirming that these are
unlikely to be natural groups. The most interesting unaddressed questions may relate to Isospora,
which has the fewest available sequences and host-parasite relationships apparently not as
straightforward as elsewhere within the suborder.

Nair, S., A. Brockman, et al. (2002). "Rapid genotyping of loci involved in antifolate drug resistance in

http://www.sciencedirect.com/science/article/B6T7F-45HFG08-1/2/a2565d108ef6242b94245d9ce5939489

Current methods used to genotype point mutations in Plasmodium falciparum genes involved in
resistance to antifolate drugs include restriction digestion of PCR products, allele-specific
amplification or sequencing. Here we demonstrate that known point mutations in dihydrofolate
reductase and dihydropteroate synthase can be scored quickly and accurately by single-
nucleotide primer extension and detection of fluorescent products on a capillary sequencer. We
use this method to genotype parasites in natural infections from the Thai-Myanmar border. This
approach could greatly simplify large-scale screening of resistance mutations of the type required
for evaluating and updating antimalarial drug treatment policies. The method can be easily
adapted to other P. falciparum genes and will greatly simplify scoring of point mutations in this
and other parasitic organisms.

http://www.sciencedirect.com/science/article/B6T7F-4447KS1-9/2/1a6c4f8e7ec7a3eb7a427c8aef5f1393

Cyclospora cayetanensis is a coccidian parasite which causes severe gastroenteritis in humans. Molecular information on this newly emerging pathogen is scarce. Our objectives were to assess genetic variation within and between human-associated C. cayetanensis and baboon-associated Cyclospora papionis by examining the internal transcribed spacer (ITS) region of the ribosomal RNA operon, and to develop an efficient polymerase chain reaction- (PCR)-based method to distinguish C. cayetanensis from other closely related organisms. For these purposes, we studied C. cayetanensis ITS-1 nucleotide variability in 24 human faecal samples from five geographic locations and C. papionis ITS-1 variability in four baboon faecal samples from Tanzania. In addition, a continuous sequence encompassing ITS-1, 5.8S rDNA and ITS-2 was determined from two C. cayetanensis samples. The results indicate that C. cayetanensis and C. papionis have distinct ITS-1 sequences, but identical 5.8S rDNA sequences. ITS-1 is highly variable within and between samples, but variability does not correlate with geographic origin of the samples. Despite this variability, conserved species-specific ITS-1 sequences were identified and a single-round, C. cayetanensis-specific PCR-based assay with a sensitivity of one to ten oocysts was developed. This consistent and remarkable diversity among Cyclospora spp. ITS-1 sequences argues for polyparasitism and simultaneous transmission of multiple strains.


http://www.sciencedirect.com/science/article/B6T7F-3Y9MD96-8/2/9d2dbb1f81a712e843e67db23c8dbbf5

mRNA and genomic DNA were isolated from adult Cyclicocyclus nassatus, and the mRNA was reverse transcribed. The cDNA was PCR amplified using degenerate primers designed according to the alignment of the [beta]-tubulin amino acid sequences of other species. To complete the coding sequence, the 3' end was amplified with the 3'-RACE, and for amplification of the 5' end the SL1-primer was used. The cDNA of the [beta]-tubulin gene of C. nassatus spans 1429 bp and encodes a protein of 448 amino acids. Specific primers were developed from the cDNA sequence to amplify the genomic DNA sequence and to analyse the genomic organisation of the [beta]-tubulin gene. The complete sequence of the genomic DNA of the [beta]-tubulin gene of C. nassatus has a size of 2652 bp and is organised into nine exons and eight introns. The identities with the exons of the gru-1 [beta]-tubulin gene of Haemonchus contortus range between 79% and 97%.


http://www.sciencedirect.com/science/article/B6T7F-42G0KDC-2/2/31f30b33e9cf8e290ebaf8419b7e64c

http://www.sciencedirect.com/science/article/B6T7F-476HJBR-1SW/2/25a4daa26419b9ff7080bb6e465587ef

The entire 1766 bases of the 18S rRNA gene of Strongyloides stercoralis have been sequenced. The gene has a 38% G + C content. Although it is similar in length to the 18S rRNA gene of Caenorhabditis elegans, the only other completely sequenced nematode 18S rRNA gene, it is only 69% identical. Closely related helminths will need to be sequenced in order to delineate sequences specific for the diagnosis of strongyloidiasis.


http://www.sciencedirect.com/science/article/B6T7F-43YSX81-F/2/8be602ce0205e12ea60d83d67bd61a6f

This study represents the first [beta]-tubulin sequence from a trematode parasite, namely, the liver fluke, Fasciola hepatica. PCR of genomic DNA showed that at least one [beta]-tubulin gene from F. hepatica contains no introns. A number of amino acids in the primary sequence of fluke tubulin are different from those described previously in various nematode species and the cestode, Echinococcus multilocularis. [beta]-Tubulin is an important target for benzimidazole anthelmintics, although (with the exception of triclabendazole) they show limited activity against F. hepatica. The amino acid differences in fluke [beta]-tubulin are discussed in relation to the selective toxicity of benzimidazoles against helminths and the mechanism of drug resistance.


http://www.sciencedirect.com/science/article/B6T7F-4C0HSD4-1/2/bacc711af1e5a70bc40cb7fcb502e92b

This is the first report of cutaneous leishmaniasis in kangaroos where infection was acquired within Australia. The diagnosis is based on the clinical criteria used for humans, the lesion histopathology, the detection and isolation of parasites from the lesions, and the analysis of the small subunit ribosomal RNA genes using the polymerase chain reaction. Despite a clear indication that the parasites belong to the genus Leishmania, no assignation to a known Leishmania species could be made using these or other less conserved genetic loci such as the non-transcribed spacer of the mini-exon repeat. As is the case in humans, some but not all animals harbouring lesions had antibodies to the isolated parasites or to several other Leishmania species. The isolated parasites displayed two well characterised Leishmania glycoconjugates, the lipophosphoglycan and proteophosphoglycan. They were infectious for mouse macrophages in vitro and established long-term infection at 33 [deg]C but not at 37 [deg]C. Our findings raise the possibility of transmission to humans, which may be unrecognised and suggest the possibility that imported species of Leishmania could become endemic in Australia.

A role for parasite genetic variability in the spectrum of Chagas disease is emerging but not yet evident, in part due to an incomplete understanding of the population structure of Trypanosoma cruzi. To investigate further the observed genotypic variation at the sequence and chromosomal levels in strains of standard and field-isolated T. cruzi we have undertaken a comparative analysis of 10 regions of the genome from two isolates representing T. cruzi I (Dm28c and Silvio X10) and two from T. cruzi II (CL Brener and Esmeraldo). Amplified regions contained intergenic (non-coding) sequences from tandemly repeated genes. Multiple nucleotide polymorphisms correlated with the T. cruzi I/T. cruzi II classification. Two intergenic regions had useful polymorphisms for the design of classification probes to test on genomic DNA from other known isolates. Two adjacent nucleotide polymorphisms in HSP 60 correlated with the T. cruzi I and T. cruzi II distinction. 1F8 nucleotide polymorphisms revealed multiple subdivisions of T. cruzi II: subgroups IIa and IIc displayed the T. cruzi I pattern; subgroups IId and Ile possessed both the I and II patterns. Furthermore, isolates from subgroups IId and Ile contained the 1F8 polymorphic markers on different chromosome bands supporting a genetic exchange event that resulted in chromosomes V and IX of T. cruzi strain CL Brener. Based on these analyses, T. cruzi I and subgroup IIb appear to be pure lines, while subgroups IIa/IIc and IId/Ile are hybrid lines. These data demonstrate for the first time that IIa/Iic are hybrid, consistent with the hypothesis that genetic recombination has occurred more than once within the T. cruzi lines.


The initiation of mucin-type O-glycosylation is catalysed by a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (EC 2.4.1.41). These enzymes are responsible for the transfer of N-acetylgalactosamine from the nucleotide sugar donor, UDP-GalNAc, to the hydroxyl group on specific serine or threonine residues in acceptor proteins. By screening a Toxoplasma gondii cDNA library, three distinct isoforms of the ppGalNAc-T gene family were cloned. Two additional isoforms were identified and partially cloned following analysis of the T. gondii genome sequence database. All of the cloned and identified ppGalNAc-T's are type II membrane proteins that share up to 50% amino acid sequence identity within the conserved catalytic domain. They each contain an N-terminal cytoplasmic domain, a hydrophobic transmembrane domain, and a luminal domain; the latter consists of stem, catalytic, and lectin-like domains. Moreover, each of this ppGalNAc-T's contains important sequence motifs that are typical for this class of glycosyltransferases. These include a glycosyltransferase 1 motif containing the DXH sequence, a Gal/GalNAc-T motif, and the CLD and QXW sequence motifs located in [alpha]-, [beta]-, and [gamma]-repeats present within the lectin-like domain. The coding regions of T. gondii ppGalNAc-T's are type II membrane proteins that share up to 50% amino acid sequence identity within the conserved catalytic domain. They each contain an N-terminal cytoplasmic domain, a hydrophobic transmembrane domain, and a luminal domain; the latter consists of stem, catalytic, and lectin-like domains. Moreover, each of this ppGalNAc-T's contains important sequence motifs that are typical for this class of glycosyltransferases. These include a glycosyltransferase 1 motif containing the DXH sequence, a Gal/GalNAc-T motif, and the CLD and QXW sequence motifs located in [alpha]-, [beta]-, and [gamma]-repeats present within the lectin-like domain. The coding regions of T. gondii ppGalNAc-T1, -T2, and -T3 reside in multiple exons ranging in number from 6 to 10. Our results demonstrate that mucin-type O-glycosylation in T. gondii is catalysed by a multimember gene family, which is evolutionarily conserved from single-celled eukaryotes through nematodes and insects up to mammals. Taken together, this information creates the basis for future studies of the function of the ppGalNAc-T gene family in the pathobiology of this apicomplexan parasite.
A reverse-transcriptase polymerase chain reaction (PCR) procedure was used to isolate an Ostertagia circumcincta partial cDNA encoding a protein with general primary sequence features characteristic of members of the mitochondrial processing peptidase (MPP) subfamily of M16 metallopeptidases. The structural relationships of the predicted protein (Oc MPPX) with MPP subfamily proteins from other species (including the model free-living nematode Caenorhabditis elegans) were examined, and Northern analysis confirmed the expression of the Oc mppx gene in adult nematodes.


Wolbachia endosymbiotic bacteria are widespread in filarial nematodes and are directly involved in the immune response of the host. In addition, antibiotics which disrupt Wolbachia interfere with filarial nematode development thus, Wolbachia provide an excellent target for control of filariasis. A 63.1 kb bacterial artificial chromosome insert, from the Wolbachia endosymbiont of the human filarial parasite Brugia malayi, has been sequenced using the New England Biolabs Inc. Genome Priming System(TM) transposition kit in conjunction with primer walking methods. The bacterial artificial chromosome insert contains approximately 57 potential ORFs which have been compared by individual protein BLAST analysis with the 35 published complete microbial genomes in the Comprehensive Microbial Resource database at The Institute for Genomic Research and in the NCBI GenBank database, as well as to data from 22 incomplete genomes from the DOE Joint Genome Institute. Twenty five of the putative ORFs have significant similarity to genes from the [alpha]-proteobacteria Rickettsia prowazekii, the most closely related completed genome, as well as to the newly sequenced [alpha]-proteobacteria endosymbiont Sinorhizobium meliloti. The bacterial artificial chromosome insert sequence however has little conserved synteny with the R. prowazekii and S. meliloti genomes. Significant sequence similarity was also found in comparisons with the currently available sequence data from the Wolbachia endosymbiont of Drosophila melanogaster. Analysis of this bacterial artificial chromosome insert provides useful gene density and comparative genomic data that will contribute to whole genome sequencing of Wolbachia from the B. malayi host. This will also lead to a better understanding of the interactions between the endosymbiont and its host and will offer novel approaches and drug targets for elimination of filarial disease.


The gene structure of a cathepsin L from Fasciola gigantica was characterised. The gene spans approximately 2.0 kb and comprises four exons and three introns and is a compact gene as in the
cases of crustaceous and platyhelminth cathepsins L. Southern blot analysis suggested that a few copies of the genes are sparsely organised in the genome. Of the three intron insertion positions, two of which are in the same position as in the mammalian cathepsin L gene. Phylogenetic analysis revealed that F. gigantica cathepsin L forms a clade with those from Fasciola hepatica, but not with those from Spirometra erinacei and schistosomes. Putative TATA-boxes were found upstream of a transcription initiation site. The sequence analysis of the 5'-upstream of the transcript revealed that the cathepsin L gene is transcribed by cis-splicing fashion. Furthermore, the experiments using recombinant F. gigantica procathepsin L showed that it was processed to an enzymatically active cathepsin L by pH-dependent autocatalysis. However, the pro-peptide deleted cathepsin L showed no enzyme activity, indicating that the pro-region of F. gigantica procathepsin L is essential for the folding and/or refolding of functional cathepsin L. These results are consistent with the observations in mammalian cathepsin L and papain.

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http://www.sciencedirect.com/science/article/B6T7H-46YV8PF-S/2/55e61eda2a011758c81dd840deef8f68

Mutations within the pncA gene coding for pyrazinamidase of Mycobacterium tuberculosis can cause pyrazinamide (PZA) resistance. The effect of drug concentrations on PZA resistance in a clinical isolate of M. tuberculosis was studied in vitro. Serial passage at gradually increased concentrations of PZA from 200 to 500 [mu]g/ml was performed using BACTEC radiometric method. Thirteen in vitro-selected variant strains were assembled and sequence analysis showed that 12 of the 13 variants had a novel single point mutation within the pncA gene by deletion at nucleotide 381 (G), codon 127. This lead to a frameshift that affected the function of the pyrazinamidase resulting in PZA resistance regardless of different PZA concentrations used. One variant had a silent mutation at nucleotide 6 (G->A) and remains PZA sensitive. We conclude that the mutation location found is an important position for full resistance, at least in this strain. The lack of further mutations even after exposure to higher PZA concentrations implies a critical value for development of resistance—a level exceeded in tissues in clinical treatment regimes.


http://www.sciencedirect.com/science/article/B6T7H-4BG8VG4-8/2/26d734046a4577db21d827846accf128

The objective of this study was to determine MICs of antibiotics for two reference strains of Coxiella burnetii using real time quantitative PCR. The method was very sensitive and specific and allowed the evaluation of the doubling time of Nine Mile and Q212 strains: 37 and 15 h, respectively. Dose response curves of antibiotics were used to determine MICs. Those of doxycycline, fluoroquinolone compounds and rifampicin were in the range 1-4 mg/l. Telithromycin was the most effective macrolide compound with MICs of 1-2 mg/l. The results confirmed
previous reports on the accuracy of this new method for the determination of the antibiotic susceptibility of C. burnetii and could be used for the screening of new drugs.


http://www.sciencedirect.com/science/article/B6T7H-4BY3YK8-2/2/9a3f6dc6a13a3945f4afdc2acc0ff39e

Klebsiella pneumoniae 2207, from Durban, was resistant to cefoxitin and [beta]-lactamase inhibitor combinations as well as oxyimino-aminothiazolyl cephalosporins. [beta]-Lactamases with isoelectric points of 5.4, 5.6, 7.6, 8.2 and 8.4 were found. DNA hybridisation identified two BamHI and three HindIII fragments carrying blaTEM, and two Sall fragments carrying blaSHV. At least two genes encoded TEM-1 enzyme; one blaSHV copy encoded SHV-5 but the other determined SHV-23, a novel SHV-5 variant with conservative amino-acid substitutions far from the catalytic site. The pI 8.4 activity was an AmpC-type enzyme. Determinants of the pI 5.6 and 7.6 activities were not identified.


http://www.sciencedirect.com/science/article/B6T7H-4DFT4GY-3/2/0eb2a51fe8e31ee1ebf3d1bfa74c4409

Immunocompromised renal transplant recipients are susceptible to severe cytomegalovirus (CMV) infection that makes its detection important in clinical practice. A total of 536 blood and 536 serum samples from 67 renal transplant recipients who had previously been diagnosed with terminal renal insufficiency were studied for CMV infection. In all samples, serology, shell vial culture, antigenaemia and nested polymerase chain reaction (PCR) in blood and serum were tested, and a real-time quantitative PCR was run on 90 specimens. Sixty-seven blood donors were used as controls. The results show that the quantitative real-time PCR assay could be of great interest for predicting CMV disease, and to monitor the onset of pre-emptive therapy.


http://www.sciencedirect.com/science/article/B6T7H-3XJK9KC-5/2/00edacef5c06fab050f68f689682d1a

One hundred and seventy-three Streptococcus pneumoniae strains isolated from surveillance studies conducted in daycare centres were studied. The mefE, erm and tet(M) genes were detected in 16.2, 45.1 and 47.4% of isolates respectively. Agreement between PCR results and antibiotic susceptibility patterns was 100%. Macrolide resistance was due to the presence of erm in 73.6% of strains and to the presence of mefE in the remaining 26.4%. All tetracycline resistant strains carried the tet(M) gene. erm was associated with tet(M) in 98.7% of strains, whereas no isolate carrying mefE carried tet(M). A significant association was found between mefE and serogroup 6 (Perm and tet(M)) and serogroup 19 (P<0.0001).

http://www.sciencedirect.com/science/article/B6T7H-427JWG1-8/2/8d6f89991b153f14c792316d5f6ca780

At our institution, isolation rates of clinical strains of ESBL-producing *Proteus mirabilis* increased to 8.8% of all *P. mirabilis* isolates during the period 1997-1999. To evaluate the susceptibility of ESBL-producing *P. mirabilis* strains against commonly used drugs, we studied 50 non-duplicated isolates selected on the basis of synergy between clavulanate and [beta]-lactams (ceftazidime, aztreonam, cefotaxime, and ceftriaxone). The presence of ESBL-coding genes was confirmed by colony hybridization with blaTEM-1 and blaSHV-1 probes. Minimum inhibitory concentrations of several antimicrobial agents for each isolate were obtained using the Etest method. All strains were encoding for TEM-derived enzymes. Gene sequencing showed that at least three different genes (TEM-15, TEM-20, and TEM-52) were present. These enzymes have not been previously reported in *P. mirabilis*. Isolates were characterized by: (a) reduced susceptibility or resistance to third- and fourth-generation cephalosporins (MIC>=2 mg/l), (b) resistance to piperacillin that was abolished by tazobactam (MIC>=256 vs. *P. mirabilis* by third-generation cephalosporins has been repeatedly observed both at our Institution and elsewhere. Piperacillin-tazobactam, as well as amikacin and meropenem appear to be important therapeutic options for infections due to multidrug-resistant, ESBL-producing *P. mirabilis* isolates.


http://www.sciencedirect.com/science/article/B6T7H-4BG8VG4-1/2/9c2389a9c2f7cc1fd0f938230ef3adf0

It has been previously demonstrated that some antimicrobial agents enhance activities of human polymorphonuclear neutrophils (PMNs). The effect on the release of cytokines in an inflammatory context from PMNs by these antibiotics was evaluated. We studied the effect of the release of some cytokines by human PMNs RT-PCR analysis on a clinical strain of *Klebsiella pneumoniae* by comparing the effect with that observed in the presence of co-amoxiclav, sanfetrinem, clarithromycin, prulifloxacin and tobramycin. All the drugs tested were capable of modulating PMN synthesis in vitro of pro-inflammatory cytokines IL-8, IL-1[beta], TNF-[alpha] and IL-6, but not that of anti-inflammatory cytokine IL-10. The degree of their stimulatory or inhibitory potency varied with the cytokine examined.


http://www.sciencedirect.com/science/article/B6T7H-41PP1PH-5/2/88795c988a63bafe8a58a7b54ece8d7

In 1996, 19 isolates of serotype 6B *Streptococcus pneumoniae* with a unique resistance pattern were found in carriers attending daycare centres in Patras, Southwestern Greece. These isolates
were penicillin susceptible but resistant to chloramphenicol, tetracycline, erythromycin, clindamycin and trimethoprim-sulphamethoxazole. Subsequently, isolates with the same characteristics were found in 23 additional carriers in central and southern Greece in 1997-98 as well as in 19 carriers in central Italy in 1997, and in seven carriers in southern Israel in 1998. Carriers were all children under 6 years of age, attending daycare centres or outpatient hospital visits. The relatedness of the isolates was determined on representative isolates from the three countries by pulsed-field gel electrophoresis of Smal digests of chromosomal DNA. Most Greek isolates were identical to each other, while isolates from Italy and Israel showed one to three band differences, with all isolates being closely related to each other as well as to the isolates from Greece. We have therefore documented the presence of this unique clone of S. pneumoniae in these three countries and have named this the ‘Mediterranean’ clone. While isolates appear to have a common origin, their source and direction of spread are unknown. However, isolates from Italy showed the most diversity, suggesting that this clone had been present in that country for a longer period than it had been in Greece.


http://www.sciencedirect.com/science/article/B6T7H-3W2YDRV-7/2/752744041523a645f19cffb8e8d56e42

The objective of the study was to evaluate a rapid screening technique for the presence of mutations in the viral reverse transcriptase gene of HIV following prolonged therapy with zidovudine in patients with AIDS. Peripheral blood mononuclear cells (PBMCs) of 14 HIV-infected patients were analyzed by micro-titer point mutation assay (PMA) before therapy with zidovudine and after at least 10 months of treatment. In addition, five of these were analyzed longitudinally. Three nontreated HIV-seropositive individuals were tested as controls. To confirm the validity of the PMA, patients' material was also analyzed with the single strand conformational polymorphism (SSCP) assay. After 10-55 months of treatment, at codons 41, 70, and 215 a shift from predominantly wild type strains to a mixture of wild type and mutant strains (21%-100% mutant sequences) appeared in the majority of patients' PBMCs. At codons 67 and 219, the wild type strain persisted after therapy in all but 3 patients. Most mutations were detected by SSCP as well as by PMA, except for one mutation at codon 41 and one at codon 70. However, when the two mutations were both present, SSCP and PMA were both able to detect these mutations. In conclusion, both PMA and SSCP are rapid and simple methods for screening for mutations causing drug resistance in zidovudine-treated HIV-infected patients. Although PMA is more labor-extensive than SSCP, the advantage of PMA over SSCP is that it permits the quantitative detection of point mutations coding for zidovudine resistance. The application of these assays may improve procedures of monitoring and modifying antiretroviral therapy on an individual basis.


http://www.sciencedirect.com/science/article/B6T7H-4BN0J57-1/2/a3a0003f9ddbdccc223221a93bad3fc4

A Klebsiella pneumoniae strain was isolated from a sputum specimen of a patient in the intensive care unit in 1999 in Shanghai Huashan Hospital, China. The isolate was confirmed as an extended-spectrum [beta]-lactamase (ESBL) producing strain by double-disk synergy test. The results of susceptibility test showed that it was resistant to most [beta]-lactams (including third
generation cephalosporins) and non-[beta]-lactam antimicrobial agents. Transconjugants were obtained at a frequency of 10^{-4}. A plasmid of about 60 kb was obtained from the transconjugant by plasmid extraction. Three major nitrocefin-hydrolysing bands with pI's of 5.4, 8.2 and 8.4, were shown in extracts of the transconjugant. Partial gene amplification products of blaTEM, blaSHV, and CTX-M-1 group gene were obtained from the isolate as well as its transconjugant. The entire blaTEM, blaSHV, and blaCTX-M in the transconjugant were amplified by PCR and the PCR products were cloned into a pHSG398 vector. Afterwards, the susceptibility of transformants and activities of [beta]-lactamases of transformants on antibiotics were tested. The PCR products were directly sequenced, analysed and identified as TEM-1, SHV-12, and CTX-M-3 genes. These results confirm that this strain of Klebsiella pneumoniae produces SHV-12, CTX-M-3 ESBLs and TEM-1 [beta]-lactamase, encoded by one single plasmid, which is responsible for the resistance of this strain to most [beta]-lactams.

**International Journal of Biochemistry** (2)


http://www.sciencedirect.com/science/article/B73GR-47F1F5V-8D/2/27ed9fbdbd44295dfdf57f2a70dbca30

1. 1. A DNA fragment encoding the [beta] subunit of bovine inhibin was amplified using the polymerase chain reaction and was cloned in plasmids pUC8 and pUR291. 2. Cultures of Escherichia coli TG2 harbouring pKDK37, a pUR291-derived recombinant plasmid, produced a novel protein with a molecular weight of 130,000 corresponding to a [beta]-galaetosidase-inhibin [beta] fusion protein. 3. The fusion protein was purified from inclusion bodies by solubilization in 8 M urea followed by an ion-exchange and gel permeation chromatography. 4. Analysis by immunoblotting and competitive radioimmuno assay revealed that the fusion protein was recognized by a monoclonal antibody raised against a chemically synthesized peptide for amino acid residues from +82 to +114 of the [beta] subunit of the bovine inhibin thereby confirming its identity.


http://www.sciencedirect.com/science/article/B73GR-47F1T6V-FX/2/4a48790111eb36fac91fe9e538cf337

1. The polymerase chain reaction has been used to amplify specifically the cDNA coding for the secreted form of ovine trophoblast protein-one from a preparation of total cellular RNA extracted from sheep embryos removed from ewes 16 days after mating. 2. Cloning and sequencing of the amplified cDNA revealed two new sequence variants: SPW49 having 93% similarity with deduced amino acid sequences from published cDNA data, and SPW27 a variant coding for a deleted form of ovine trophoblast protine-one. 3. The gene for ovine trophoblast protein-one is intronless. 4. This study provides further evidence for the existence of an ovine trophoblast protein-one gene family. 5. Both variants contain a potential N-glycosylation site not apparent in published sequences for ovine trophoblast protein-one.

http://www.sciencedirect.com/science/article/B6T16-40GHY30-6/2/88ebcb0e484e4939d8353a4ec0175863

It has been suggested that a Q/R (Gln192Arg) polymorphism of paraoxonase (PON) might be associated with the predisposition to coronary artery disease (CAD). Therefore, we studied the human paraoxonase gene (PON1) polymorphism in Turkish patients with CAD by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). This polymorphism was determined in 96 CAD patients and in 105 control subjects. The frequencies of the QQ, QR, and RR genotypes were found as 36.5, 52.0, and 11.5% in CAD patients and 48.6, 41.0, and 10.4% in control subjects, respectively. The QR genotype was the most common in the patient group, whereas the QQ genotype was more frequent in individuals without CAD. Frequency of the R allele was higher among CAD patients compared to controls (38.5% versus 31%). However, neither the genotype nor the allele distribution of the Gln92Arg polymorphism of PON1 was statistically significantly different between the two groups (P>0.05). Although both systolic and diastolic blood pressure levels were slightly higher in patients with the QQ genotype, there was no differences in regard to age, sex, serum triglyceride, total cholesterol or high-density lipoprotein cholesterol among CAD patients with different PON1 Gln192Arg genotypes. In summary, our results suggest that no association exists between the Gln192Arg polymorphism of paraoxonase and CAD in Turkish patients.


http://www.sciencedirect.com/science/article/B6T16-4BC2MBR-C/2/a228bddd12892566add0bd43079db64d4

Background: Abnormalities of collagen and elastic fibers were found in floppy mitral valves (FMV). Urokinase-plasminogen activator (PLAU) was suggested to be involved in the pathogenesis of elastin and collagen degradation in arterial aneurysm. The role of PLAU genetic variant in mitral valve prolapse (MVP) has not been studied. We, therefore, performed a case-controlled study investigating the possible relation between the PLAU gene polymorphisms and risk of MVP in Taiwan Chinese. Methods: We studied 100 patients with MVP diagnosed by echocardiography and 106 age- and sex-matched normal control subjects. The T4065C and T3995C polymorphisms of the PLAU gene were identified by polymerase chain reaction (PCR)-based restriction analysis. Results: There was a significant difference in either the genotype distribution or allelic frequencies between MVP cases and controls for PLAU gene T4065C polymorphism (P=0.0001 and 0.0002, respectively). An odds ratio for risk of MVP associated with PLAU T4065C TC genotype was 6.03 (95% confidence interval 2.11-14.83). An odds ratio for risk of MVP associated with PLAU T4065C T allele was 4.99 (95% confidence interval 1.93-12.91). There was no significant difference in either the genotype distribution or allelic frequencies between MVP cases and controls for PLAU T3995C polymorphism. Further categorization of the
MVP patients into mild and severe subgroups revealed no statistical difference between these two subgroups for PLAU T4065C and T3995C polymorphisms. Conclusions: This study shows that patients with MVP have a higher frequency of PLAU T4065C TC genotype and T allele that supports a role of the PLAU T4065C polymorphism in determining the risk of MVP among the Chinese population in Taiwan.


http://www.sciencedirect.com/science/article/B6T16-414NWXF-D/2/db7443b01ae41493013ce530ba9b798b

To further investigate the immunological mechanisms involved in Takayasu's arteritis, we analyzed the T-cell receptor (TCR) V[gamma] and V[delta] gene usage by infiltrating [gamma][delta] T-cells and the expression of costimulatory molecules B7-1, B7-2, CD40, CD27 ligand (CD27L), CD30L, OX40L in the arterial tissue of a patient with Takayasu's arteritis. We found that the repertoires of TCR V[gamma] as well as V[delta] gene transcripts of the infiltrating cells were restricted as compared with those of peripheral blood lymphocytes from a patient with Takayasu's arteritis. This strongly suggests that [gamma][delta] T-cells as well as [alpha][beta] T-cells, as we previously reported, were specifically involved in the pathogenesis of Takayasu's arteritis. We also found that B7-1, B7-2, CD40, CD27L, CD30L, and OX40L were expressed in the arterial tissue, suggesting the roles for these costimulatory molecules in T-cell-mediated vascular injury in Takayasu's arteritis. Our findings strongly support the involvement of T-cell-mediated immunological mechanisms in the pathogenesis of Takayasu's arteritis.


http://www.sciencedirect.com/science/article/B6T16-472BNT6-9/2/1d2261c603199b29384bcefa515528c4

Background: Proinflammatory cytokines such as tumor necrosis factor [alpha] (TNF-[alpha]), interleukin (IL)-6, and IL-8 have been implicated in myocardial injury following cardiopulmonary bypass (CPB). However, little evidence is currently available to directly confirm such a relationship. We have previously documented that a newly discovered 'four and a half LIM-only protein 2' (FHL2) is exclusively expressed in myofibres. We hypothesized that the upregulation of FHL2 is proportional to the degree of myocardial injury and investigated the myocardial expression of FHL2 together with these cytokine messenger RNAs (mRNAs) during clinical CPB. Methods: Intermittent hypothermic blood cardioplegia was used in all patients. Atrial myocardial biopsies were obtained immediately at the onset and at the end of CPB in 33 consecutive patients undergoing valvular or coronary artery surgery. TNF-[alpha], IL-6, and IL-8 mRNA expressions in these myocardial samples were determined by semi-quantitative reverse transcription-polymerase chain reaction. Myocardial FHL2 expression was determined by Western blot analysis. Serum levels of the MB isoenzyme of creatine kinase (CK-MB) and cardiac troponin-I (cTnI) before surgery and 24 h after the end of CPB were also measured. Results: The duration of aortic crossclamping and CPB was 70+/-33 and 99+/-37 min, respectively. No elevated myocardial TNF-[alpha] mRNA expression was found after CPB. IL-6 mRNA expressions were detected in 14 pairs of the myocardial biopsies and were elevated in 11 (33%) post-CPB biopsies. Similarly, IL-8 mRNA expressions were detected in 19 pairs of samples and were elevated in 14 (42%) post-CPB biopsies. Among the 17 pairs of biopsies with positive FHL2 expression, FHL2 levels were increased in 11 (33%) post-CPB samples. Moreover, the elevated
FHL2 expression was associated with an increase in IL-6 (P=0.018) and IL-8 (P=0.024) mRNA expression after CPB. Postoperative CK-MB and cTnI levels were significantly higher in patients with myocardial FHL2 expressions than those without (CK-MB, 13.5+/−2.3 vs. 6.5+/−0.8 ng/ml, P=0.022; cTnI, 10.7+/−2.0 vs. 3.5+/−0.6 ng/ml, P=0.0013). Conclusions: Our findings demonstrate for the first time that both IL-6 and IL-8 mRNAs are upregulated in human cardiac myocytes following CPB and these cytokines may be involved in myocardial ischemia-reperfusion injury, as reflected by their association with an increased expression of FHL2.


http://www.sciencedirect.com/science/article/B6T01-4D4PP9T-3/2/cf76390d5224a34a2a4d68d345b799f

The molecular mechanisms of [beta]-amyloidogenesis in sporadic Alzheimer's disease are still poorly understood. To reveal whether aging-associated increases in brain oxidative stress and inflammation may trigger onset or progression of [beta]-amyloid deposition, a transgenic mouse (Tg2576) that express the Swedish double mutation of human amyloid precursor protein (APP) was used as animal model to study the developmental pattern of markers of oxidative stress and APP processing. In Tg2576 mouse brain, cortical levels of soluble [beta]-amyloid (1-40) and (1-42) steadily increased with age, but significant deposition of fibrillary [beta]-amyloid in cortical areas did not occur before postnatal age of 10 months. The slope of increase in cerebral cortical [beta]-secretase (BACE1) activities in Tg2576 mice between ages of 9 and 13 months was significantly higher as compared to that of the [alpha]-secretase, while the expression level of BACE1 protein and mRNA did not change with age. The activities of superoxide dismutase and glutathione peroxidase in cortical tissue from Tg2576 mice steadily increased from postnatal age 9-12 months. The levels of cortical nitric oxide, and reactive nitrogen species demonstrated peak values around 9 months of age, while the level of interleukin-1[beta] steadily increased from postnatal month 13 onwards. The developmental temporal coincidence of increased levels of reactive nitrogen species and antioxidative enzymes with the onset of [beta]-amyloid plaque deposition provides further evidence that developmentally and aging-induced alterations in brain oxidative status exhibit a major factor in triggering enhanced production and deposition of [beta]-amyloid, and potentially predispose to Alzheimer's disease.


http://www.sciencedirect.com/science/article/B6T01-485H1WS-2/2/38cdab6e701af6a3e801a9a4f2eaf96d

Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3) are structurally related survival and differentiation factors for distinct sets of peripheral and central neurons. We previously reported that BDNF and NGF gene expression are differentially regulated
in mouse L929 fibroblasts. Here we examine expression of these three neurotrophins in human fibroblasts. Northern blots detected BDNF and NT-3 mRNAs in fibroblasts derived from lung (WI-38), calvarium and foreskin. WI-38 cells and foreskin fibroblasts expressed 1.6 kb as well as 4 kb BDNF mRNAs whereas only the smaller BDNF mRNA was detected in calvarium fibroblasts. NGF mRNA was present in foreskin and calvarium but not lung fibroblasts. In WI-38 cells serum treatment increased levels of BDNF mRNA within 2 hr. Cycloheximide did not inhibit the increase. Treatment with 12-O-tetradecanoyl phorbol-13-acetate (TPA) transiently suppressed BDNF mRNA. Treatment with both serum and TPA first stimulated and then transiently suppressed BDNF mRNA. TPA and/or serum did not significantly affect BDNF mRNA in calvarium fibroblasts. These results show that human fibroblasts derived from different tissues express and regulate neurotrophin genes differentially.


http://www.sciencedirect.com/science/article/B6T01-485YMB3-D7/2/ca48a3edec97b9dfc4754002c040b182

The actions of the neurotrophins are mediated through specific receptors. Nerve growth factor (NGF), the prototypic neurotrophin, binds to receptors of both high and low affinity. A protein 75 kDa in size (p75NGFR) binds NGF, as well as brain-derived neurotrophic factor and neurotrophin 3, with low affinity. Recent investigations suggest that this protein may also be a component of the high affinity NGF receptor complex. To study gene expression of the p75NGFR molecule, we used a sensitive reverse transcription-polymerase chain reaction (RT-PCR) assay to measure levels of its messenger RNA (mRNA) in small samples of total RNA. The assay is based on using a shortened p75NGFR cRNA as an internal RNA standard to control for variability in reverse transcription and polymerase chain amplification. We measured p75NGFR mRNA levels in the rat cerebellum during ontogeny to further study the transient developmental increase in receptor gene expression known to occur in this brain region during the early postnatal period. We found that p75NGFRmRNA levels were most abundant at postnatal day 2, and then declined to lower levels throughout postnatal development and in the adult. Northern blot analysis of the same total RNA samples used in our RT-PCR assay verified that p75NGFR expression is highest in the early postnatal period. These results confirm those of previous studies accomplished with much larger amounts of RNA using ribonuclease protection or northern blot assays. The use of an RT-PCR assay that utilized an internal standard also controls against changes in RNA complexity which can affect the measurement of message abundance across developmental stages. These results again suggest that p75NGFR may play an important role in the postnatal development of cerebellum in rats.


http://www.sciencedirect.com/science/article/B6T01-41BF4B8-8/2/bacd51ef8f50f717e7ce514cb8fb30d1

In this report, we describe the effect of nerve growth factor (NGF) on the transcriptional expression of voltage-dependent Ca[2] channel [alpha]1 subunits, i.e., [alpha]1A, [alpha]1B, [alpha]1C, [alpha]1D, and [alpha]1E in rat pheochromocytoma (PC12) cells. Using reverse transcriptase-coupled polymerase chain reaction (RT-PCR) and class-specific Ca[2] channel oligonucleotide probes, messenger RNA levels were measured and compared to Histone H3.3
transcript which remained relatively constant over the duration of NGF treatment. Although no statistically significant differences in P-type ([alpha]1A) Ca[2] channel transcript levels were observed, N-type ([alpha]1B) Ca[2] channel transcript levels increased 50% over control values (P values [alpha]1C and [alpha]1D) transcripts with [alpha]1C decreasing steadily to ~50% of control (P value [alpha]1D decreased to ~20% of control (P value [alpha]1E Ca[2] channel transcripts were detected in PC12 cells. For comparison, PC12 cells were also treated with another differentiative growth factor, i.e., basic fibroblast growth factor (bFGF) and a nondifferentiative growth factor epidermal growth factor (EGF). In contrast to NGF, bFGF and EGF treatment had no inhibitory effect on L-type ([alpha]1C and [alpha]1D) channel transcript levels after 3 days. Like NGF, EGF treatment had no statistically significant effect upon P-type ([alpha]1A) transcript levels but increased in a biphasic manner following bFGF treatment. Presynaptic-associated [alpha]1B (N-type) Ca[2] channel transcripts were observed decreased following EGF treatment (2 days) while L-type [alpha]1C transcripts decreased after 7 days (P value 2] channel transcripts, while L-type ([alpha]1C and [alpha]1D) Ca[2] channel transcripts appear to be down regulated.


http://www.sciencedirect.com/science/article/B6T01-42Y117P-5/2/58874e96c26c7e7431c24303e7cc

Changes in the metabolic activity within the brain of patients suffering from Alzheimer's disease (AD) were investigated and compared with biochemical alterations in the hippocampus induced by fimbria/fornix transection in the rat. The deafferentation of the hippocampus results in a degeneration of cholinergic septo-hippocampal terminals accompanied by a persistent decrease of choline acetyltransferase (ChAT) and acetylcholine esterase (AChE) activities similar to the cholinergic malfunction in AD. In the animal model the [3H]-cytochalasin B binding to the glucose transporters was elevated up to the day 7 after surgery as was the activity of the phosphofructokinase (PFK) on day 3. A reactive astrogliosis could be evidenced by the upregulation of glial fibrillary acidic protein (GFAP). An increase of the PFK activity was also found in AD being accompanied by enhanced level of GFAP as well. A higher concentration of mRNA for all three isoenzymes of PFK was shown by reverse transcription (RT)-real time polymerase chain reaction (PCR) amplification. However, the pattern of PFK isoenzyme proteins and mRNAs did neither change in diseased human nor in the lesioned rat brain. The activities of the mitochondrial enzymes pyruvate dehydrogenase complex (PDHC) and cytochrome c oxidase (CO) were diminished in the lesioned rat hippocampus on day 7 as well as in AD brain. Subcellular fractionation showed that the activity of these enzymes was affected in the synaptosomal as well as in the extrasynaptosomal mitochondria indicating a loss of neuronal input and also a vulnerability of intrinsic hippocampal neurons and/or non-neuronal cells. The recovery of the mitochondrial enzyme activity in the animal model at later post lesion intervals may be the result of compensatory responses of surviving cells or of sprouting of other non-affected inputs. It is concluded that common metabolic mechanisms may underlie the concurrent degenerative and repair processes in the denervated hippocampus and the diseased Alzheimer brain.


http://www.sciencedirect.com/science/article/B6T01-3PFC5W0-10/2/60ac13b42314526097b6efabcaaa0980c
The growth and differentiation of olfactory sensory neurons are regulated tightly. We had shown previously, by immunohistochemistry, that transforming growth factor-[alpha] (TGF-[alpha]) and epidermal growth factor (EGF) receptor are present in the olfactory epithelium of untreated adult rats and that TGF-[alpha] is a potent mitogen of olfactory epithelium in vitro. Expression of EGF receptor and TGF-[alpha] was detected primarily in horizontal basal cells and supporting cells but rarely in globose basal cells, which suggested that EGF receptor is not a likely candidate for the mitotic regulator of sensory neurons. In order to expand the search for candidate regulators, we have now examined other members of the EGF family of receptors and ligands. By utilizing reverse transcriptase-polymerase chain reaction (RT-PCR) methodology, we have detected the messenger RNA encoding the protein of the neu gene (p185neu) and Neu differentiation factor (NDF) isoforms in the olfactory mucosa. Immunohistochemical localization of p185neu and NDF indicates expression of these proteins in the olfactory epithelium of adult rats in regions where globose basal cells and immature sensory neurons are found, as well as in the ensheathing cells of the olfactory nerve. The presence of neu and NDF transcripts in the olfactory tissue and the localization of their encoded polypeptides to proliferative regions of the epithelium suggest involvement of these gene products in the regulated proliferation/differentiation of the sensory neurons.


http://www.sciencedirect.com/science/article/B6T01-3PFC5W0-T/2/b169d7a80af5560330831ad15c555a9f

Voltage-gated sodium channels are responsible for the initial depolarizing phase of the action potential. In hippocampal neurons cultured from trisomy 16 (Ts16) mice (a model for Down's syndrome), the maximum inward conductance mediated by these channels was reduced 47% relative to control diploid neurons. This reduced conductance was reflected in a 35% decrease in binding of radiolabeled saxitoxin, a sodium channel-specific ligand, indicating expression of fewer channels in these neurons. The mRNAs encoding the [alpha] and [beta]1 subunits were, however, present at the same levels in Ts16 neurons and control diploid neurons. Thus, the altered regulation of voltage-gated sodium channels in Ts16 neurons is apparently a post-transcriptional event and possible mechanisms are discussed.

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http://www.sciencedirect.com/science/article/B6T7K-49CRCXW-1/2/f1d4b44b29539b7ae1e79abb7c3a4e7

The incidence of Listeria monocytogenes in a vegetable processing plant was investigated over a 23-month period. Frozen ready-to-eat vegetable samples, as well as the plant environment, were sampled. The molecular subtyping techniques, Random Amplified Polymorphic DNA (RAPD) and
Restriction Endonuclease Analyses (REA), were performed to help investigate the origin and routes of Listeria dissemination. The low and sporadic incidence of L. monocytogenes made it impossible to establish an epidemiological sequence in the processing plant, though a case of cross-contamination between tomato and ratatouille was detected. Listeria innocua subtyping, however, allowed us to determine the prevalence of several strains in vegetables, and their presence on machinery samples suggested the possibility of cross-contamination during processing. The low incidence of L. monocytogenes indicated that the risk of listeriosis transmission by vegetable consumption is low. On the other hand, the isolation of the same strain of L. innocua in several surveys pointed out the risk of colonisation on surfaces and machinery. The persistence of Listeria spp. is a cause for concern as can lead to future contamination of vegetables processed in the plant and to a possible increased risk for health. Therefore, periodic controls for the presence of Listeria spp. and a further review of the cleaning and disinfection procedures used in frozen vegetable plants are recommended.


http://www.sciencedirect.com/science/article/B6T7K-4C7DF6C-2/2/834a5ddbb98ea14555c4d07119c3970c

Wine grapes harvested at different stages during cultivation from several vineyards in New South Wales, Australia, harboured Bacillus thuringiensis at viable populations of 102-106 cfu/g. Commercial preparations of B. thuringiensis had been sprayed onto the grapes as a biological insecticide. B. thuringiensis (101-103 cfu/ml) was isolated from grape juice and fermenting grape juice in a commercial winery. Although B. thuringiensis remained viable when inoculated at 103-104 cfu/ml into grape juice and wine (pH 3.0-6.0), it did not grow. Using in vitro agar culture assays, B. thuringiensis inhibited several grape-associated yeasts and bacteria as well as various species of fungi associated with grape spoilage and ochratoxin A production. B. thuringiensis did not inhibit Saccharomyces cerevisiae in agar culture or during alcoholic fermentation of grape juice. B. thuringiensis inhibited the malolactic bacterium, Oenococcus oeni, in agar culture but not during mixed cultures in a liquid medium.


http://www.sciencedirect.com/science/article/B6T7K-4771XB6-1K/2/cc0f4d4ab46fdcc062c61fb668836c2

Techniques for the identification of the spoilage yeasts Saccharomyces cerevisiae and members of the Zygosaccharomyces genus from food and beverages sources were evaluated. The use of identification systems based on physiological characteristics resulted often in incomplete identification or misidentification. Also the cellular fatty acid analysis failed on differentiating species within the Zygosaccharomyces genus. However, the Random Amplified Polymorphic DNA (RAPD) assay, using selected 10-mer oligonucleotides, allowed discrimination between all species tested. For this RAPD assay, a simple and reproducible method of DNA isolation from spoilage yeast cells is described.

Fresh produce has been repeatedly implicated as the source of human viral infections, including infection with hepatitis A virus (HAV). The objective of the present study was to evaluate the HAV adsorption capacity of the surface of various fresh vegetables that are generally eaten raw and the persistence of the HAV. To this end, the authors experimentally contaminated samples of lettuce, fennel, and carrot by immersing them in sterile distilled water supplemented with an HAV suspension until reaching a concentration of 5 log tissue culture infectious dose (TCID50)/ml. After contamination, the samples were stored at 4 \(^\circ\)C and analysed at 0, 2, 4, 7, and 9 days. To detect the HAV, RT-nested-PCR was used; positive samples were subjected to the quantitative determination using cell cultures. The three vegetables differed in terms of their adsorption capacity. The highest quantity of virus was consistently detected for lettuce, for which only a slight decrease was observed over time (HAV TITRE=4.44\(\pm\)0.22 log TCID50/ml at day 0 vs. 2.46\(\pm\)0.17 log TCID50/ml at day 9, before washing). The virus remained vital through the last day of storage. For the other two vegetables, a greater decrease was observed, and complete inactivation had occurred at day 4 for carrot and at day 7 for fennel. For all three vegetables, washing does not guarantee a substantial reduction in the viral contamination.


Highly similar gene sequences of the 5' region of the large subunit (LSU) are commonly interpreted to predict the organism's identity. However, it was recognised that closely related taxa do not always show sufficiently diverged D1/D2 LSU sequences to differentiate between them. The effectiveness of species separation using D1/D2 LSU sequences, small subunit (SSU) sequences and actin gene sequences was determined by pair-wise comparisons. The LSU data showed coinciding similarities among and within species. The actin data resolved all investigated species. Examples strengthened the value of almost complete SSU sequences for species separation. The larger number of differences in the highly conserved actin gene, compared to the overall more variable LSU gene, is due to the tolerance of protein coding genes to synonymous nucleotide changes. In contrast, the pairing in secondary structures of the rRNA, ensuring the functionality of the molecule, relies on longer and uninterrupted sequence sections. In conclusion, D1/D2 LSU sequences are not specific enough to identify closely related taxa. The actin gene is a better marker in these cases. However, because of the availability of a large database of fungal D1/D2 LSU sequences, this gene region is currently still the preferred target for sequence-based identification.


Oysters harvested in western France, from five sites associated with outbreaks of food-borne norovirus gastroenteritis between February 2000 and March 2001, were assayed for enterovirus RNA by reverse transcriptase-heminested polymerase chain reaction (RT-heminested PCR). Forty percent (21/52) of shellfish samples (pool of seven oysters) were contaminated by
enteroviruses. Infectious coxsackieviruses serotype A21 were isolated from three of these positive samples. Amplicons corresponding to 65 base sequences in the 5' untranslated region of the enteroviral genome were analyzed by direct sequencing. Interpretable results were obtained from 18 amplicons, but mixtures of sequences confused the results from 3 samples. Sequences isolated from samples from the different sites were different but similarities were observed between sequences detected in shellfish from two sites at different dates. Sequences were also compared to sequences of human, bovine and porcine enteroviruses. Both human and animal origins of enterovirus contamination of shellfish seemed likely.


Eighty-nine strains of Lactobacillus delbrueckii subsp. lactis isolated from Italian hard and semi-hard cheeses and artisan starter cultures were characterised by phenotypic and genotypic methods. Phenotypic diversity was evaluated by studying biochemical characteristics (i.e. acidifying and peptidase activities) of technological interest. Genotypic diversity was evidenced by RAPD-PCR and pulsed field gel electrophoresis (PFGE). Phenotypic characterisation indicated a wide variability of the acidifying activity within Lact. delbrueckii subsp. lactis. Although the data was variable, it allowed us to evidence groups of strains with different acidifying properties, especially in terms of acidification intensity. Concerning peptidase activity, Lact. delbrueckii subsp. lactis showed a homogeneously high x-prolil-dipeptidil-aminopeptidase activity and a considerable but more heterogeneous lysil-aminopeptidase activity. The increased resolution obtained by the use of two molecular typing techniques, i.e. RAPD-PCR and PFGE, allowed to widen the level of strain heterogeneity. Technological and ecological pressures are determinant in selecting Lact. delbrueckii subsp. lactis sub-populations which are more functional to the different cheese technologies.


Thirty-five strains of Lactobacillus delbrueckii subsp. lactis and subsp. bulgaricus isolated from dairy products were typed by restriction fragment length polymorphism (RFLP) of protein-coding genes. The strains were analysed by RFLP of PCR amplified, infragenic fragments of the following housekeeping genes: [beta]-galactosidase, lactose permease, and proline dipeptidase. Sequencing of the variable regions of the 16S rDNA was then performed on a reduced number of strains. PCR-RFLP analysis evidenced wide strain heterogeneity. Strains were grouped into genotypes according to both subspecies assignment and infra-species genetic polymorphism. This polymorphism was related to the presence of microbial groups within subspecies populations. The low infra-species sequence polymorphism detected in the variable region of the 16S rRNA gene did not enable to group the strains with the same sensitivity reached by PCR-RFLP of protein-coding genes. PCR-RFLP of protein-coding genes applied to L. delbrueckii seems a promising tool to evaluate microbial diversity within bacterial subpopulations. Differences among bacterial subpopulations based upon molecular heterogeneity in protein-coding genes would enable to better understand the role of strains from different ecological niches.

http://www.sciencedirect.com/science/article/B6T7K-4771XB6-1N/2/6de766b0c80ea8cfbc8f9ef6d3a84b3e

Detection of the coccoid form of Campylobacter jejuni with the use of the polymerase chain reaction (PCR) was examined. Coccoid cells of this pathogen, formed at different temperatures, showed different detection characteristics in the PCR. For spirals and cocci formed at 4[deg]C and 12[deg]C, the detection limit was about 2 x 10^3 cells/PCR. However, for detection of coccoid cells formed at 25[deg]C and 37[deg]C, at least 2 x 10^4 cells per PCR were needed. PCR was also performed on homogenates in peptone saline solution and enrichment broths of chicken meat and chicken liver that were artificially contaminated with cocci formed at 4[deg]C. PCR-products of these samples could not be demonstrated clearly.


http://www.sciencedirect.com/science/article/B6T7K-42BSPWM-7/2/6b9c2c361d579c26771a787fb5cb14f7

The presence of genes for the production of the three components of the HBL enterotoxin complex and enterotoxin-T in Bacillus cereus was evaluated by PCR tests for strains isolated from milk. In addition enterotoxin production of B. cereus was evaluated by means of the HBL blood agar plate and two commercially available toxin tests. All three genes for the HBL enterotoxin complex were detected in 55% of the 86 strains tested, the enterotoxin-T gene was detected in 62% of the strains. A few strains showed a weak reaction in the PCR tests for the L1 or L2 components of the HBL enterotoxin complex. Many strains that were found to contain the genes for the HBL complex gave negative or doubtful results in the HBL blood agar plate test. All strains that contain the L2 part of the HBL complex showed a titer of at least 8 in the Oxoid RPLA test. Two strains that did not contain the L2 part of the HBL enterotoxin complex gave high titers (=64) in the RPLA test.


http://www.sciencedirect.com/science/article/B6T7K-3W2T595-7/2/8a2b407e57f67cb118b26779c95dd2b

The induction of interferon (IFN) and interleukin-1 (IL-1) production in murine macrophages by a phosphopolysaccharide, produced by a dairy lactic acid bacteria, Lactococcus lactis ssp. cremoris, was investigated. When the phosphopolysaccharide was added into macrophage cultures at concentrations from 1 to 200 [mug/ml, substantial IFN titers (6.2-79.2 IU/ml) were detected. Using the reverse transcription-polymerase chain reaction (RT-PCR), the expression of mRNA encoding IFN-[gamma] was verified in spleen macrophage cultures. Macrophages
stimulated with the phosphopolysaccharide also produced IL-1[alpha] at a concentration of 50 [mu]g/ml. This study showed for the first time that phosphopolysaccharide derived from a dairy lactic acid bacterium can induce IFN-[gamma] and IL-1[alpha] production in macrophages. These findings strongly suggest that the phosphopolysaccharide is a type of 'biological response modifier' and the fermented dairy foods containing Lactococcus lactis ssp. cremoris can be designated as a physiologically functional food.


http://www.sciencedirect.com/science/article/B6T7K-4C9HPBB-1/2/6c39cb6b3592d31fca3eac0b5ecadaad

Phylogenetic relationships between four Fusarium species were studied using parts of the nuclear translation elongation factor-1 alpha (EF-1[alpha]) gene as a phylogenetic marker. Sequences from 12 isolates of Fusarium poae, 10 isolates of Fusarium sporotrichioides and 12 isolates of Fusarium langsethiae yielded 4, 5 and 5 haplotypes, respectively. In addition, we included one isolate of Fusarium kyushuense. The aligned sequences were subjected to neighbor-joining (NJ), maximum parsimony and maximum likelihood (ML) analyses. The results from the different analyses were highly concordant. The EF-1[alpha]-based phylogenies support the classification of F. langsethiae as a separate taxon in the section Sporotrichiella of Fusarium, as the closest sister taxon to F. sporotrichioides, while F. kyushuense is the sister taxon to F. poae. This corresponds well with the ability of F. langsethiae and F. sporotrichioides to produce T-2 and HT-2 toxins. In contrast, morphological characters indicate a closer relationship between F. langsethiae and F. poae on the one hand, and between F. sporotrichioides and F. kyushuense on the other hand.


http://www.sciencedirect.com/science/article/B6T7K-4DS98YD-2/2/452ab637395bb8e22e53d536f97f855d

The morphological variation, secondary metabolite profiles and restriction fragment length polymorphisms (RFLPs) of PCR amplified intergenic spacer (IGS) ribosomal DNA (rDNA) were studied in 27 isolates of Fusarium equiseti, 25 isolated from Norwegian cereals and 2 from soil obtained from the IBT culture collection (BioCentrum, Technical University of Denmark). All 27 isolates were tested for production of fusarochromanone (FUSCHR), zearalenone (ZEA) and the trichothecenes: 15-monoacetoxy-scirpentriol (MAS), diacetoxy-scirpenol (DAS), T-2 and HT-2 toxins, T2-triol, neosolaniol (NEO), deoxynivalenol (DON), nivalenol (NIV) and 4-acetylnivalenol (Fus-X). The trichothecenes were analysed by GC-MS in a selected ion monitoring mode, while FUSCHR was determined by ion pair HPLC with fluorometric detection and production of ZEA by TLC. For amplification of IGS rDNA primers CNL12 and CNS1 were applied. IGS rDNA was digested with the four restriction enzymes: AvaII, CfoI, EcoRI and Sau3A. In addition, we sequenced the IGS rDNA region of three of the Norwegian isolates. There were two morphological types among the Norwegian strains of F. equiseti, type I with short apical cells (dominating) and type II with long apical cells, with four haplotypes identified based on the RFLP data. Variation in secondary metabolite profiles within and between the morphological groups was observed and the levels of produced toxins were: FUSCHR 3000-42,500 and 25-30 ng/g, NIV 20-2500 and 120-700 ng/g, FUS-X 20-15,000 and 0 ng/g, DAS 30-7500 and 0-600 ng/g, and MAS
10-600 and 0-500 ng/g, for strains with short and long apical cells, respectively. NEO was detected in 16/27 strains tested (all morphotype I). All but four strains of type I (these four lacked a restriction site for EcoRI) had identical RFLP profiles. The isolates of type II had two haplotypes. The IGS sequence similarity data indicated differences between these morphotypes corresponding to two separate lineages apparently at the species level.


http://www.sciencedirect.com/science/article/B6T7K-3RJNRBN-9/2/5a9d03d4a417f6e9363d85ea0e20b4c4

A sample treatment method based on buoyant density centrifugation which separates bacteria from food, concentrates bacteria and removes PCR inhibitors is described. The method involves a one minute centrifugation of food homogenate layered over a gradient medium (Percoll(R) or BacXtractor(TM)) in Eppendorf tubes, followed by a single wash step. The small scale of this treatment makes it possible to process many samples in a short time. To evaluate the method beef and minced beef samples, spiked with strains of Escherichia coli O157:H7, were treated and then analysed by PCR aimed at verocytotoxin- (VT1 and VT2) and eae-genes. The detection limits in 1:10 (w/v) beef and minced beef homogenates were 125-250 cfu ml-1 (1250-2500 cfu g-1) and 1000 cfu ml-1 (1 x 104 cfu g-1), respectively. The enrichment of spiked samples in buffered peptone water at 37 [deg]C for 6 hours before buoyant density centrifugation and PCR, allowed 0.5 cfu g-1 beef and 5 cfu g-1 minced beef to be detected. This combination of enrichment and buoyant density centrifugation was also used for analysis of 43 beef samples from a consignment in which E. coli O157:H7 had been detected, and detected VT-genes in all 43 samples. E. coli O157:H7 was also separated and detected in spiked samples of milk, lettuce, shrimps, and blue cheese at arbitrary concentrations of 3000 cfu ml-1. The present sample preparation method has the potential to be applicable to many other combinations of bacteria and food, and in connection with other detection methods than PCR as well.


http://www.sciencedirect.com/science/article/B6T7K-44724S4-9/2/a7d857954d81811dcd6306131ae18b98

The non-conjugative 46 kb plasmid that encodes the biosynthesis of lacticin 3147 in Lactococcus lactis IFPL105 has been transferred to the starter L. lactis IFPL359, used in goat's milk cheesemaking. The accelerating effect exerted on proteolysis and development of sensory characteristics of semi-hard cheese by the bacteriocin-producing transconjugant L. lactis IFPL3593 (Lac+ Bac+ Imm+), which is able to induce cell lysis in starter adjuncts with high peptidase activity, has been studied. It has been demonstrated that the use of IFPL3593 as starter accelerates cheese ripening as it increases the level of amino nitrogen correlated with early cell lysis of adjuncts. The fact that the bacteriocin-producing microorganism used is immune to the bacteriocin, allowed proper acidification of the curd without altering the cheese-making process.

Mayer, Z., A. Bagnara, et al. (2003). "Quantification of the copy number of nor-1, a gene of the aflatoxin

http://www.sciencedirect.com/science/article/B6T7K-46FXSDH-2/2/3e8843db592d5e80a0a098dade336983

A real-time PCR system directed against the nor-1 gene of the aflatoxin biosynthetic pathway as a target sequence has been applied to detect an aflatoxinogenic *A. flavus* strain in plant-type foods like maize, pepper and paprika. The system is based on the TaqMan(R) fluorescent probe technology. The copy numbers of the nor-1 gene were compared to conventional cfu data obtained from the same set of samples. In general, a good correlation between nor-1 gene copy number and the cfu data was observed; however, the nor-1 copy numbers were always higher. It was shown that the system is specific for nor-1 containing species.


http://www.sciencedirect.com/science/article/B6T7K-3VCVFHR-8/2/96c11d3ef685c609690190b4b4a2243152

The presence of virulence genes, encoding enterohemorrhagic *Escherichia coli* (EHEC)-hemolysin (EHEC-hlyA), intimin (eae), and Shiga toxins 1 (stx1) and 2 (stx2), in 178 isolates of pathogenic *E. coli*, was determined using the polymerase chain reaction with primers specific for each virulence gene. The tested organisms were 120 isolates of *E. coli* O157:H7 from human patients, cattle, sheep and foods, 16 non-O157:H7 EHEC isolates from patients suffering from hemorrhagic colitis or hemolytic uremic syndrome, 15 non-O157:H7 Shiga toxin-producing *E. coli* (STEC) isolates from cattle and foods, 26 isolates of enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterotoxigenic *E. coli* (ETEC), and an *E. coli* K12 strain. Results revealed that all isolates of O157:H7 carried EHEC-hlyA, eae, and one or both stx genes; 15 of the 16 non-O157:H7 EHEC isolates carried EHEC-hlyA, but all possessed eae and one or both stx genes; only seven of the 15 non-O157 STEC isolated from cattle and foods contained both EHEC-hlyA and eae genes. The EPEC, EIEC, ETEC, and the *E. coli* K12 strain did not carry these virulence genes, except eight EPEC isolates were positive for eae. Results suggest that a combination of EHEC-hlyA and eae genes could serve as markers to differentiate EHEC from less pathogenic STEC, and other pathogenic or non-pathogenic *E. coli*.


http://www.sciencedirect.com/science/article/B6T7K-3W2T5DW-1B/2/144f0e6429266a27fbb52545ede63d0792

*Escherichia coli* O157:H7 is known as an important cause of hemorrhagic colitis and hemolytic uremic syndrome. Real-time procedures that are sensitive for detecting small populations of this bacterium in food are lacking and needed. An expression library was constructed by ligation of BamHI-EcoRI DNA fragments of *E. coli* O157:H7 to plasmid vector pUC19 and transformation of recombinant plasmids to *E. coli* JM109. A clone that contained a specific DNA fragment of *E. coli* O157:H7 was identified by colony immunoblot assay using monoclonal antibody MAb 4E8C12 that uniquely links to *E. coli* O157:H7 and a few other serotypes of verotoxin-producing *E. coli*. The DNA sequence of the clone consisted of 110 bp of 5' region of enterohemorrhagic *E. coli* (EHEC) eae gene and a 688 bp DNA fragment adjacent to 5' end of the eae gene, including an
unknown function gene encoding 156 amino acids. A pair of oligonucleotide primers was synthesized based on the sequence of the 688 bp fragment. The primers were used in a polymerase chain reaction (PCR) to amplify a target DNA of 633 bp. The primers amplified 1 ng of DNA from 67 strains of E. coli O157:H7, two strains of E. coli O157:NM, and 7 of 11 E. coli O55:H7 and O55:NM strains, but not 50 ng of DNA from 34 strains of 29 other E. coli serotypes and 25 strains of 8 other bacterial species. Annealing temperatures from 60 to 63 [deg]C could be used for the PCR without loss of specificity. The minimum amount of target DNA detected by the PCR was 5 pg. When a boiling method and GeneReleaser were used, the PCR was able to detect as few as 25 and 38 CFU of E. coli O157:H7, respectively, in 3 h.


Ten commercially available pesticides (insecticides, herbicides and fungicides), used during the production of vegetable produce, were examined as potential sources of microbial contaminants. As purchased, none of the pesticides showed the presence of viable microorganisms (Pseudomonas, Salmonella and Escherichia coli. Listeria monocytogenes did not survive after inoculation into any of the pesticides. Pesticides were reconstituted in different sources of agricultural water (bore, dam and river) and examined for survival and growth of microorganisms naturally present in these waters. On storage at 30 [deg]C for 48 h, nine of the pesticides supported the growth of bacterial species present in these waters. Predominant species in the pesticide solutions, before and after storage, varied according to the source, but species of Pseudomonas, Acinetobacter and Aeromonas and various coliforms exhibited significant growth. Unless managed properly (reconstituted in potable water, and used without lengthy storage), pesticides could contribute to the microbial load of vegetable produce, thereby affecting their shelf-life and public health safety.


Advances in detection and quantification assays based on nucleic acids conceivably will revolutionize the ability to quickly and specifically detect and quantify microorganisms in foods. Among these assays, the polymerase chain reaction (PCR) assay and the TaqMan(TM) PCR Detection System (Perkin-Elmer) probably are among the most promising. Since a 5'-nuclease PCR renders possible the automated and direct detection and quantification of PCR products (Holland et al., 1991. Proc. Natl. Acad. Sci. USA 88, 7276-7280), microorganisms in foods can be detected and quantified indirectly within a few hours through analysis of the microbial DNA or RNA sequences present. In the present report we have adapted a 5'-nuclease-based kit for the quantification of Salmonella.

Following consumption, stomach acidity is the first major barrier encountered by the food-borne pathogen Listeria monocytogenes. Analysis of low pH sensitivity and glutamate decarboxylase (GAD) acid resistance system of 14 isolates of L. monocytogenes carried asymptomatically by humans showed that levels of GAD activity were subjected to strain variation. Similar variations were observed for strains responsible for 18 cases of listeriosis, whereas in comparison, 13 strains isolated from food and food-processing plant environments showed lower GAD activity.

Following survival of the stomach barrier, L. monocytogenes also has to resist bile salts encountered in the small intestines. Analysis revealed that all strains tested were able to grow in the presence of bile salts with concentrations as high as those encountered in the small intestines and had previously identified bile salt hydrolase (BSH) activity. Strain variation was observed but there was no relationship between the origin of the strains and the ability to degrade bile salts.


Microbiological sampling of Norvegia cheese from three cheese factories was done during ripening. The evolution of aerobic mesophilic bacteria, lactococci, lactobacilli, enterococci, presumptive leuconostoc and pediococci was investigated after 30, 90, 180 and 270 days of ripening. Isolates (135) of non-starter lactic acid bacteria (NSLAB) from nine Norvegia cheeses after 90, 180 and 270 days of ripening were examined. The isolates were tested by physiological and biochemical assays, species-specific PCR and 16S rDNA sequencing. After 90 days of ripening Leuconostoc sp., most probably from the starter, and the NSLAB specie Lactobacillus paracasei dominated among the isolates, however, after longer ripening Lb. paracasei dominated. The development and evolution of the microflora in Norvegia varied according to dairy and ripening time.


Rapid identification of filamentous fungi is becoming increasingly important in food mycology both for monitoring the production process and for the identification of food spoilers. This paper describes the development and trial of two specific PCR primer sets. A 336 bp fragment from species belonging to Penicillium subgenus Penicillium was amplified by the primers ITS 212d and ITS 549. The other primer set, ITS 183 and ITS 401 specifically identified two species. Penicillium roqueforti and P. carneum, both known as spoilers in the bread industry, by amplification of a 300 bp fragment. The future perspectives of PCR based identification of filamentous fungi in food are discussed.

A completely selective enrichment procedure was compared with two partially nonselective ones for the detection of *Listeria monocytogenes* in cheeses. After enrichment for approximately 48 h, the enrichment media were streaked on selective agars and presumptive *Listeria* colonies were confirmed using PCR. In some cases, PCR was also performed directly on the enrichment broth. The conventional, completely selective enrichment procedure was not always the best choice for the detection of stressed *L. monocytogenes* in cheeses. Especially in the case of semi-hard cheeses from pasteurized milk and soft cheeses of the blue veined and the red smear types, the methods that incorporated a nonselective enrichment step gave better results than the completely selective method. For mold ripened, soft cheeses, the results were highly dependent on the brand of cheese and time of sampling, but the best results were obtained using the completely selective enrichment procedure.


The rapid detection of an average of 5.9 stressed Salmonella cells in 25 g of food product using immunomagnetic separation (IMS) and PCR is described. For pasteurised egg yolk, egg yolk powder, ice-cream, whole egg, egg white and cheeses made from pasteurised milk PCR was applied after 16 h of preenrichment in buffered peptone water (BPW) using IMS and alkaline lysis as sample preparation method. For whole egg and egg white the BPW was supplemented with iron. For milk powder, and raw milk cheeses, the 16-h preenrichment in BPW was followed by IMS and a 4-h enrichment in Rappaport-Vassiliadis broth. In the latter case, PCR was applied on the enrichment medium after centrifugation and alkaline lysis. For PCR the primers ST11 and ST15 (Aabo et al., 1993) producing a fragment of 429 bp were used. An internal PCR control, designed to be co-amplified with the target DNA using the same primers but producing a smaller fragment of 240 bp, was used.


LightCycler(TM) technology combines rapid in vitro amplification of DNA with real time detection and quantification of the amount of target molecules present in a sample. The system enables a 35-cycle PCR with 32 samples do be completed in 45 min, including quantification and identification of the product. It is therefore well suited for routine analysis of large numbers of samples in quality control and for defining HACCP concepts. Based on PCR primers specific to the tri5 gene, a quantitative group specific assay was established for Fusarium species producing trichothenes. In the assay, SYBR(R)Green I was used as fluorescent dye enabling real time detection of PCR products. Characterisation of the amplicons was achieved by melting point
analysis (85+/−0.1 [deg]C). Nonspecific products such as primer dimers could readily be distinguished from the product by their lower melting points. Composition of the amplification buffer was optimised and various hot start methods were tested in order to achieve the highest sensitivity of the assay. Uracil DNA glycosylase was added to prevent amplification of nonspecific products due to DNA carryover. The spectrum of species detected was generally in accordance with the results found in conventional PCR using the Tox5 primer pair. Reproducibility in six parallel experiments of the assay was determined to be 98% in the range between 0.05 and 6 ng of purified Fusarium graminearum DNA. The assay was used to analyse 30 wheat samples contaminated with toxigenic Fusarium spp. Contamination ranged from 0% to 78% as revealed by mycological analysis, and this is compared with results from the LightCycler(TM). This is the first report on the use of the LightCycler(TM) system in combination with SYBR(R)Green I for the quantification and identification of fungal DNA in pure cultures and sample material.


http://www.sciencedirect.com/science/article/B6T7K-47DTB1D-1/2/ff5280e2554f7c4ae1fb199ab987b3f4

Four hundred and seventy-two generic Escherichia coli isolates were recovered from ground and whole retail beef, chicken, pork, and turkey obtained from Greater Washington, DC, USA during the years 1998 to 2000. Many of the isolates displayed resistance to tetracycline (59%), sulfamethoxazole (45%), streptomycin (44%), cephalothin (38%) and ampicillin (35%). Resistance was also observed, but to a lesser extent, to gentamicin (12%), nalidixic acid (8%), chloramphenicol (6%), ceftiofur (4%) and ceftriaxone (1%). Sixteen percent of the isolates displayed resistance to one antimicrobial, followed by 23% to two, 23% to three, 12% to four, 7% to five, 3% to six, 2% to seven and 2% to eight. Three E. coli isolates were shown to possess Shiga toxin genes (stx2) via PCR; all were O non-typeable and were recovered from ground beef samples purchased on the same day at the same supermarket. One of the Shiga toxin-producing E. coli (STEC) isolates was susceptible to each of the antimicrobials tested, whereas one displayed resistance to cephalothin and sulfamethoxazole, and one displayed resistance to ampicillin, cephalothin, gentamicin, streptomycin, sulfamethoxazole and tetracycline. Findings from this study indicate that retail raw meats may often be contaminated with antimicrobial-resistant E. coli.


http://www.sciencedirect.com/science/article/B6T7K-409VGKY-7/2/6895857ade009fe1a3c5f2b2fbb8adb

A combined method based on traditional culturing, buoyant density centrifugation, (BDC), and polymerase chain reaction (PCR) techniques for detection and identification of pathogenic Y. enterocolitica in food was developed and evaluated. An internal control, which was added in each PCR-tube and co-amplified by the same primer pair as the pathogen, monitored false-negative PCR results. The sample preparation step, BDC, was used to remove PCR inhibiting food substances and to concentrate the Y. enterocolitica cells. Single PCR with a chromosomal gene (ail) as target was chosen for screening the samples. The method was tested on naturally and artificially contaminated food samples. In three different food samples, processed meat (brawn), unprocessed beef and minced pork, inoculated with 10 cfu pathogenic Y. enterocolitica per gram,
Y. enterocolitica was detected and cultural bacteria indicated within 18 h of enrichment.


http://www.sciencedirect.com/science/article/B6T7K-42M1DDR-B/2/ec2a457be279e0ec1c0f9be146a5c07e

One hundred and forty-eight Listeria monocytogene isolates originating from vacuum packed cold-smoked salmon produced in 10 different Danish smokehouses were compared by Random Amplified Polymorphic DNA (RAPD) profiling. A total of 16 different reproducible RAPD profiles were obtained using a standardised RAPD analysis by four primers separately. The grouping of the 148 strains was exactly the same for the four primers used. For a sub-set of 20 strains typed by Pulsed Field Gel Electrophoresis (PFGE), only one strain was allocated into a different group as compared to the grouping by RAPD typing. Different RAPD types dominated in products from different smokehouses. Some identical RAPD types were isolated in several smokehouses. In each of four smokehouses, one particular RAPD type could be repeatedly isolated from products. Each smokehouse/product carried its own specific RAPD type and this may indicate a possible persistence of closely related strains of L. monocytogenes in smokehouses.


http://www.sciencedirect.com/science/article/B6T7K-3W2V4C8-7/2/02bcc956526001f75b5517ef60abfd6f

As part of a WHO multicenter study on Listeria monocytogenes subtyping methods the random amplification of polymorphic DNA (RAPD)-technique was evaluated. Six participants were asked to use a standard protocol to analyse a set of 80 L. monocytogenes strains. This set contained 22 groups of epidemiologically linked isolates and 11 pairs of duplicate strains. Using three different 10-mer primers, the median reproducibility of the RAPD-results obtained by the six participants was 86.5% (range 0-100%). Failure in reproducibility was mainly due to results obtained with one particular primer. The number of epidemiological groups found to be homogeneous varied from 1-22 (median 16). However, for some groups an inhomogeneity was found by the majority of participants. The overall correlation between the results from the different participants ranged from 32 to 85%.

International Journal of Gynecology & Obstetrics (1)


http://www.sciencedirect.com/science/article/B6T7M-405SVBF-
Objective: Thalassemia is a highly prevalent genetic disorder in Taiwan. The major goal of this study was to present a feasible protocol for the prenatal diagnosis of thalassemia. Method: Prenatal investigation of thalassemia was performed on 57 at-risk cases at the Mackay Memorial Hospital, Taipei, Taiwan. We developed a method using polymerase chain reaction (PCR) and high-throughput DNA sequencing to detect mutations. All diagnoses were confirmed after delivery. Result: Prenatal testing revealed 16 normal fetuses, 24 [alpha]-thal-1 carriers, eight Hb Bart's hydrops fetalis, seven [beta]-thalassemia minor, and two [beta]-thalassemia major fetuses. No false-positive or false-negative cases were found during the postnatal follow-ups. Conclusion: The results of this study indicate that prenatal diagnosis of thalassemia syndromes in Taiwan is successful with the use of a rapid and accurate molecular method.

International Journal of Infectious Diseases (2)


http://www.sciencedirect.com/science/article/B7CPT-4BNT1X7-3S/2/804965c361d431f48569da9e4b400e12

Objectives: To investigate the incidence and epidemiology of non-multiresistant methicillin-resistant Staphylococcus aureus (nmMRSA) infection in south-east Queensland, Australia. Study design: A retrospective survey was done of hospital records of all patients who had non-multiresistant MRSA isolated at Ipswich Hospital (a 250-bed general hospital, 40 km south-west of Brisbane, Queensland, Australia) between March 2000 and June 2001. Laboratory typing of these isolates was done with antibiogram, pulsed-field gel electrophoresis, bacteriophage typing and coagulase gene typing. Results: There were 44 infections caused by nmMRSA. Seventeen infections (39%) occurred in patients from the south-west Pacific Islands (predominantly Samoa, Tonga and New Zealand). Laboratory typing showed that the isolates in Pacific Islanders were Pacific Island strains, and 16/17 of these infections were community acquired. Twenty-three infections (52%) occurred in Caucasians. Eleven of the isolates from Caucasians (48%) were a new predominantly community-acquired strain that we have termed the 'R' pulsotype, nine (39%) were Pacific Island strains, and three (13%) were health care institution-associated strains. Four infections occurred in patients who were not Caucasians or Pacific Islanders. Overall, 34 of all 44 infections (77%) were community acquired. Conclusions: Non-multiresistant MRSA infection, relatively frequently observed in Pacific Islanders in south-east Queensland, is now a risk for Caucasians as well, and is usually community acquired. Clinicians should consider taking microbiological specimens for culture and antimicrobial susceptibility testing in patients with suspected staphylococcal infections who are not responding to empirical therapy with [beta]-lactam antibiotics.


http://www.sciencedirect.com/science/article/B7CPT-4BM4PWW-
Background: Atypical serum neutralizing antibody responses to prototype strains of Puumala viruses in some patients with hemorrhagic fever with renal syndrome (HFRS) have long suggested the existence of other hantaviruses in the Balkans. Objective: To determine the presence of arvicolid rodent-borne Puumala-like hantaviruses in Yugoslavia. Materials and Methods: Using reverse transcript-polymerase chain reaction, Tula virus RNA was amplified from lung tissues of a European pine vole (Pitymys subterraneus) captured in 1987, following an outbreak of HFRS in the Cacak region of Serbia-Yugoslavia. Results: Sequence analysis of the entire coding region of the S segment and a 948-nucleotide region of the G2 glycoprotein-encoding M segment revealed divergence of approximately 14% from Tula virus strains harbored by European common voles (Microtus arvalis) captured in Central Russia and the Czech Republic. However, nearly complete identity was found in the corresponding deduced amino acid sequences. Moreover, phylogenetic trees constructed by the maximum parsimony and neighbor-joining methods indicated that this Pitymys-borne hantavirus shared a common ancestry with other Tula virus strains. Conclusions: The data demonstrate that Pitymys subterraneus also serves as a rodent reservoir of Tula virus in Serbia-Yugoslavia. To what extent this represents virus spillover from Microtus arvalis warrants further investigation.

International Journal of Mass Spectrometry (2)


http://www.sciencedirect.com/science/article/B6VND-4BYN5ST-4/2/e96b5a055b5dd56b6473848998138b8eb5

A 53-base pair region on the long arm of chromosome 22 was amplified using PCR with 7-deaza-modified deoxynucleotides. Increased amplification efficiency was achieved by doubling the concentration of the modified deoxynucleotide triphosphates. Incorporation of 7-deaza purines has been previously shown to selectively eliminate fragmentation pathways during gas-phase sequencing of nucleic acids by sustained off-resonance irradiation collision-induced dissociation (SORI-CID) and infrared multiphoton dissociation. However, 7-deaza analogs result in significant duplex stability precluding interrogation of the single-stranded species by tandem mass spectrometry. Herein, we demonstrate the use of lambda exonuclease to successfully overcome this problem and are able to obtain single-stranded PCR products containing 7-deaza adenine and guanine nucleobases. Mass accuracy was used as our metric to determine complete incorporation of 7-deaza residues in PCR products>15 kDa; averatide) chemical formula developed herein which was based on the relative frequencies of AT and GC base pairs in the human genome. Single-stranded PCR products were fragmented using SORI-CID and as expected, cleavage at the 7-deaza modified sites was not observed. Collectively, this integrated approach can facilitate top-down sequencing of PCR products by a variety of tandem mass spectrometry methods.

In this work, we describe a strategy for the detection and characterization of microorganisms associated with a potential biological warfare attack or a natural outbreak of an emerging infectious disease. This approach, termed TIGER (Triangulation Identification for the Genetic Evaluation of Risks), relies on mass spectrometry-derived base composition signatures obtained from PCR amplification of broadly conserved regions of the microbial genome(s) in a sample. The sample can be derived from air filtration devices, clinical samples, or other sources. Core to this approach are "intelligent PCR primers" that target broadly conserved regions of microbial genomes that flank variable regions. This approach requires that high-performance mass measurements be made on PCR products in the 80-140 bp size range in a high-throughput, robust modality. As will be demonstrated, the concept is equally applicable to bacteria and viruses and could be further applied to fungi and protozoa. In addition to describing the fundamental strategy of this approach, several specific examples of TIGER are presented that illustrate the impact this approach could have on the way biological weapons attacks are detected and the way that the etiologies of infectious diseases are determined. The first example illustrates how any bacterial species might be identified, using Bacillus anthracis as the test agent. The second example demonstrates how DNA-genome viruses are identified using five members of Poxviridae family, whose members includes Variola virus, the agent responsible for smallpox. The third example demonstrates how RNA-genome viruses are identified using the Alphaviruses (VEE, WEE, and EEE) as representative examples. These examples illustrate how the TIGER technology can be applied to create a universal identification strategy for all pathogens, including those that infect humans, livestock, and plants.

International Journal of Medical Microbiology(3)


Intestinal infections due to shiga toxin-producing Escherichia coli bacteria (STEC) reveal a broad range of clinical symptoms and a large scale of virulence properties of the respective pathogens. The question whether all STEC variants or only a particular group of them need to be considered for clinical and epidemiological purposes was answered throughout this study. Using the PCR technique for the identification of 25 different virulence-associated genes, 266 E. coli strains belonging to 81 different E. coli serotypes from various clinical origins were investigated. A great genetic diversity of the virulence properties and a broad range of virulence marker combinations have been identified. However, distinct virulence marker combinations (e.g. Stx2/LEE/pO157 as well as Stx2dac/pO113) were found to be associated with the same notified clinical symptoms (e.g. HUS). Such an association speaks either for the "shiga toxin-only concept" or for several redundant, but clinically or epidemiologically important virulence properties.

http://www.sciencedirect.com/science/article/B7GW0-4F97397-4/2/a190909fba2cd1d814a1c80e2c231c69

Multilocus sequence typing (MLST) has become the gold standard for typing of a variety of bacterial and fungal micro-organisms. Others recently reported the successful use of the tiling DNA array technology to sequence-type Staphylococcus aureus. We now evaluated microarrays based on polymorphism-directed oligonucleotide design for typing of Neisseria meningitidis. The rationale behind this approach was to minimize the number of microarray probes by exploiting the comprehensive knowledge of polymorphisms combined in the Neisseria MLST website. Initial experiments using model oligonucleotides of 28-32 base-pairs in length revealed that the hybridization protocols used were highly specific. However, despite of several optimization steps, the rate of misidentification of oligonucleotides remained >1.8% in consecutive validation experiments using arrays representing the genetic diversity at three MLST loci. We assume that the high density of polymorphic sites and the extensive GC-content variations at N. meningitidis MLST loci hinder the successful implementation of MLST microarrays based on polymorphism-directed oligonucleotide design.


http://www.sciencedirect.com/science/article/B7GW0-4DN99XW-6/2/d1d686410420f7766874bcdd7318eed

A DNA fingerprinting method for the characterization of Legionella pneumophila serogroup 1 strains was established. This method was based on the DNA extraction using Chelex 100 and subsequent PCR analysis using primers under conditions of low stringency. Sixteen single primers were tested for the typing of the 10 epidemiologically unrelated reference strains of L. pneumophila serogroup 1 as well as patient isolates and environmental strains isolated from the water system of a hospital where patients with legionellosis were treated. In addition, a combination of two primers (Lpm-1 and Lpm-2) originally established for the specific detection of Legionella strains was tested. The PCR results were compared with two further subtyping methods, i.e. monoclonal antibody analysis and pulsed-field gel electrophoresis. The type strains Philadelphia 1, Knoxville 1, Allentown 1, Benidorm 0303E, Bellingham 1, and France 5811 could be distinguished clearly in experiments using all of the primers. Depending on the primer used, Heysham 1 and Oxford 4032E showed different DNA profiles. The strains Olda and Camperdown 1 were nearly indistinguishable. In contrast, the analysis by PFGE and MAb subtyping revealed distinct types for all 10 reference strains. The discrimination of the patient isolates from two suspected cases of nosocomial legionellosis and environmental isolates was not possible with the 16 single primers used in the study. However, the PCR assay with the combination of Lpm-1 and Lpm-2 as well as the PFGE and MAb analysis were able to differentiate distinct types. The use of the sequence-specific primers under low-stringency annealing conditions allowed both simultaneous gene detection as well as epidemiological typing of Legionella strains.
Split hand/split foot malformation (SHFM), which typically appears as lobster-like limb malformation, is a rare clinical condition caused by a partial deletion of chromosome 7q. Hearing impairment sometimes accompanies syndromic SHFM cases; a case of inner and middle ear malformation with SHFM is described in this report. We conducted a genetic evaluation of this patient and found a deleted region that overlaps a previously reported locus of SHFM as well as a DFNB14 locus that can cause nonsyndromic hearing impairment by autosomal recessive inheritance.


http://www.sciencedirect.com/science/article/B6T7V-44J3TM5-1/2/d470d1b9367bb208852c1e37e1afde26

Objective: We applied mutation screening in seven cochlear implant users to identify those persons with GJB2-related deafness to determine whether etiology of deafness was predictive of speech performance after implantation. Methods: Direct sequence of GJB2 was conducted over seven cochlear implant users with prelingual hearing impairment and their speech, language and cognitive performance was examined. Results: The three persons with GJB2-related deafness had a mean vocabulary of 1243 words compared to a mean vocabulary of 195 words in the four children with GJB2-unrelated deafness, although the number of patients examined here was limited. The developmental quotient (DQ) of cognitive ability also was higher in those children with GJB2-related deafness. Conclusions: These preliminary results suggest that better speech performance after cochlear implantation may be observed in persons with GJB2-related deafness. In the future, detailed phenotypic studies and mutation screening for non-syndromic hearing loss may play an important role in the preoperative assessment of prelingually-deafened children.


http://www.sciencedirect.com/science/article/B6T7V-40HV0MB-4/2/c83cf79cccccde8295f07a84d4a5c6ca

Apoptosis -- programmed death of a cell -- is a natural mechanism that controls the number of cells in an organism. Neoplastic cells as many types of normal cells, may be the subject of spontaneous apoptosis as well as they may be induced by anti-neoplastic factors. Neoplastic cells' resistance to drugs is often correlated with impossible induction of apoptosis in those cells. Though the process of apoptosis is not fully explained, a possible involvement of many genes in regulation of this process is indicated. One of them is bcl-2 gene and its product -- bcl-2 protein, which has the property of apoptosis process inhibition and stimulation of a cell towards outliving (survival). Increased expression of bcl-2 gene is present in many neoplastic cells and it suggests a possible pathogenic role of bcl-2 gene in oncogenesis. In this paper the expression of bcl-2...
gene in the cells of papilloma in larynx is defined in six children operated in the Department of Paediatric Otolaryngology of Medical School in Lublin. Papillomas of larynx are neoplasms of particular resistance to treatment. Complete, cellular RNA was isolated with Chomczynski and Sacchi method using guanidine thiocyanate. Gene expression was defined with the method of reverse transcription by cDNA synthesis and amplification of bcl-2 gene fragment with specific oligonucleotides in reverse transcriptase polymerase chain reaction (RT-PCR). The products were identified on agarose gel. Expression of bcl-2 gene in the investigated cells of laryngeal papilloma was confirmed in all the children. The presence of bcl-2 gene product in these cells may be the cause of apoptosis inhibition and stimulation of cells proliferation of the neoplasm.

International Journal of Radiation Oncology*Biology*Physics (7)


http://www.sciencedirect.com/science/article/B6T7X-429XX63-11/2/5904ecc7be6e656ac7b9b9d1c5dbd85a

Purpose: To investigate the correlation between tumor potential doubling time, Tpot, and mutations in the p53 gene, TP53, and the potential of these parameters to predict outcome of head and neck cancer patients treated with radiotherapy.Methods and Materials: Data from two independent studies on Tpot and TP53 mutations were combined, including 58 patients with squamous cell carcinoma of the head and neck. Tpot was estimated on biopsies obtained 6-9 h after infusion of iododeoxyuridine by combined flow cytometry and immunohistology. TP53 mutations were detected using DGGE and sequenced. All patients received primary radiotherapy alone.Results: The predictive value of Tpot alone was of borderline significance. However, in TP53 wild-type tumors, Tpot was a strong predictor of outcome, whereas Tpot in TP53 mutant tumors failed to provide any information. Tpot and TP53 were not associated with nodal control; however, there was a strong relationship with control in the T-position, disease-specific survival, and overall survival.Conclusion: Tpot can to be a relevant parameter for predicting outcome of radiotherapy in head and neck cancer but only in the subset of patients without mutations in the p53 gene.


http://www.sciencedirect.com/science/article/B6T7X-456FCPG-G/2/82b7662dcbc07c93ecb17549b923b9b6: The objective of this study was twofold: first, to identify patients with locally advanced breast cancer (LABC) who will achieve a pathological response to a preoperative regimen of concurrent paclitaxel and radiation; and second, to explore associations between molecular markers from the original tumors and pathological response.: Patients with previously untreated LABC were eligible to receive a regimen of preoperative concurrent paclitaxel, 30 mg/m2 twice a week for a total of 8 weeks, and radiation delivered Weeks 2-6, 45 Gy at 1.8 Gy per fraction to the breast, ipsilateral axilla, and supraclavicular nodes. At mastectomy, pathologic findings were classified as pathological complete response
(pCR) = no residual invasive cells in the breast and axillary contents; pathological partial response (pPR) = presence of neu and p53 overexpression. Estrogen receptor (ER), HER2/neu, metablastin, [beta]-tubulin III and IV, microtubule-associated protein-4 (MAP-4), bcl-2, bax, and cyclooxygenase-2 (COX-2) gene expression were measured using real-time quantitative polymerase chain reaction (PCR). A total of 36 patients had pretreatment biopsies and were evaluable for the analysis of the association of molecular markers with pathological response. Pathological response in the mastectomy specimen was achieved in 12 of these 36 patients (33%). Only HER2/neu and ER gene expression were found to be significantly associated with the extent of pathological response to the regimen, i.e., tumors with low HER2/neu gene expression and negative estrogen receptors were more likely to respond to the tested regimen (p = 0.009 and p = 0.006, respectively). Conversely, p53 protein expression measured by IHC did not appear to be associated with pathological response (p = 0.67). Further studies in LABC should assess whether patient selection for treatment based on the original tumor molecular characteristics could affect their chance to achieve a pathological response.


http://www.sciencedirect.com/science/article/B6T7X-3WPPR1F-G/2/13deea8a953cdef8532a5af65bf90

Purpose: To determine, retrospectively, the status of the bp 609 mutation in the DT-diaphorase gene in anal canal carcinoma patients who have undergone radical radiotherapy with concurrent 5-fluorouracil (5-FU) and mitomycin C (MMC), to determine the relationship of the mutant form of the gene to treatment outcomes. Methods and Materials: Paraffin blocks of pretreatment tumor biopsies were obtained on 49 patients who underwent treatment with curative intent using radiation, infusional 5-FU and bolus MMC from January 1991 to December 1993. DNA was extracted and subjected to polymerase chain reaction (PCR) analysis using primers that encompassed the bp 609 C to T mutation. Restriction endonuclease cleavage with Hinf 1 and gel electrophoresis were used to determine the polymorphism status of each patient. Results: DNA of 46 patients was successfully amplified. The 46 patients were distributed as follows: 26 (56.5%) C/C--homozygous wildtype, 18 (39%) T/C--heterozygous, and 2 (4.5%) T/T--homozygous mutant. Eleven of 46 patients had suffered treatment failure. The status of the bp 609 polymorphism in this group was 5 (45.5%) C/C, 5 (45.5%) C/T, and 1 (9%) T/T. Conclusion: In this series, there was not an overrepresentation of the mutant allele in patients with treatment failure, suggesting that the bp 609 alteration is not a strong determinant of treatment outcome.


http://www.sciencedirect.com/science/article/B6T7X-4F9MJMX-10/2/c06d54060c8f5c7a200e56686e6f8c8

Purpose: Bcl-2, an inhibitor of apoptosis frequently shows elevated expression in human tumors, thus resulting in resistance to radiation therapy. Therefore, inhibiting Bcl-2 function may enhance the radiosensitivity of tumor cells. Tetrocarcin A (TC-A) and bcl-2 antisense oligonucleotides exhibit antitumor activity by inhibiting Bcl-2 function and transcription, respectively. We investigated whether these antitumor agents would enhance the cytotoxic effects of radiation in tumor cells overexpressing Bcl-2. Methods and materials: We used HeLa/bcl-2 cells, a stable Bcl-2-expressing cell line derived from wild-type HeLa (HeLa/wt) cells. Cells were incubated with TC-A
and bcl-2 antisense oligonucleotides for 24 h after irradiation, and cell viability was then determined. Apoptotic cells were quantified by flow cytometric assay.

**Results**
The HeLa/bcl-2 cells were more resistant to radiation than HeLa/wt cells. At concentrations that are not inherently cytotoxic, both TC-A and bcl-2 antisense oligonucleotides increased the cytotoxic effects of radiation in HeLa/bcl-2 cells, but not in HeLa/wt cells. However, in HeLa/bcl-2 cells, additional treatment with TC-A in combination with radiation did not significantly increase apoptosis.

**Conclusions**
The present results suggest that TC-A and bcl-2 antisense oligonucleotides reduce radioresistance of tumor cells overexpressing Bcl-2. Therefore, a combination of radiotherapy and Bcl-2 inhibitors may prove to be a useful therapeutic approach for treating tumors that overexpress Bcl-2.

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**Purpose:** The dose intensity of radiotherapy (RT) used in cancer treatment is limited in rare individuals who display severe normal tissue reactions after standard RT treatments. Novel predictive assays are required to identify these individuals prior to treatment. The mechanisms responsible for such reactions are unknown, but may involve dysfunction of genes involved in the sensing and response of cells to DNA damage. The breast cancer susceptibility genes BRCA1 and BRCA2 are implicated in DNA damage repair and the control of genome stability. The purpose of this study was to determine if clinical radiation hypersensitivity is related to mutations of the BRCA1 and BRCA2 genes. Such information is of potential use in the clinical management of BRCA mutation carriers and their families.

**Methods and Materials:** Twenty-two cancer patients who developed severe normal tissue reactions after RT were screened for mutations of BRCA1 and BRCA2, using various methods including protein truncation testing, direct DNA sequencing, and a PCR-based BRCA1 exon 13 duplication test.

**Results:** No mutations were detected in the 22 patients tested, despite screening for the majority of commonly described types of mutations of BRCA1 and BRCA2.

**Conclusion:** These early results suggest that genes other than BRCA1 and BRCA2 probably account for most cases of clinical radiation hypersensitivity, and that screening for mutations of BRCA1 and BRCA2 is unlikely to be useful in predicting response to radiotherapy. However, it has not been excluded that some BRCA1 or BRCA2 heterozygotes might experience unexpected RT toxicity; further BRCA mutation screening on radiation sensitive individuals is warranted.

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**Purpose:** Spontaneous apoptosis has been shown to predict tumor response to radiochemotherapy in rectal cancer in vivo. It remains to be elucidated, however, which genetic profile determines whether a tumor is more or less prone to apoptosis. Recently, a novel member of the inhibitor of apoptosis protein family, designated survivin, was identified. We investigated the impact of surviving expression on tumor cell apoptosis in three colorectal cell lines of different intrinsic radiosensitivities.

**Methods and materials:** Survivin protein expression was measured by Western blot analysis, and survivin mRNA expression by quantitative TaqMan reverse transcriptase polymerase chain reaction (qRT-PCR) analysis.
transcription polymerase chain reaction, both in untreated cell and after irradiation with 2 and 8 Gy. The expression profile was then correlated to spontaneous and radiation-induced apoptosis (Tunel-Assay, DAPI-staining) in three colorectal cell lines of low (SW 480), intermediate (HCT-15), and high radiosensitivity (SW 48), as determined by the colony-forming assay. Results In vitro analysis revealed higher spontaneous and higher radiation-induced apoptosis rates in the radiosensitive line (SW 48), as compared with the more resistant line (SW 480). In Western blot analysis and in TaqMan analysis, SW 480 was characterized by a higher spontaneous expression and a pronounced induction of survivin 48 h after irradiation, whereas survivin expression was low when untreated and not increased after irradiation in the most radiosensitive line SW 48. HCT-15 was intermediate, both with respect to the level of survivin mRNA and protein expression.

Conclusion The inverse correlation of survivin-expression with spontaneous and radiation-induced apoptosis suggests that survivin is an important inhibitor of apoptosis in colorectal cancer cell lines. Analysis of survivin mRNA or protein expression may therefore provide predictive information on radio- and chemoresistance of individual colorectal tumors.


http://www.sciencedirect.com/science/article/B6T7X-44HTB4T-S/2/ce74ea381334016e77cab26c3ece8d20

Purpose: The aim of this study was to investigate the efficacy of combination therapy of ionizing radiation (IR) and adenoviral p53 gene therapy and to evaluate its molecular mechanisms. Methods and Materials: Two human prostate cancer cell lines, DU145 and PC-3 cells, containing different types of p53 gene mutations, were investigated. The recombinant adenovirus vector containing the wild-type p53 gene (Ad5CMV-p53) was used for this study. Cells were irradiated (in 0, 2, 4, and 6 Gy, 300 cGy/min) and after 12 h of irradiation, the cells were infected with various doses of Ad5CMV-p53 (0-40 multiplicity of infection [MOI]). Cytotoxicity was determined by clonogenic assay. The molecular mechanisms were evaluated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), apoptotic cell detection, and cell cycle analysis. Results: The cell growth inhibition in DU145 (p53-mutated) cells by IR was strongly enhanced by additional Ad5CMV-p53 infection in a viral dose-dependent manner. In DU145 cells, IR alone induced minimal p53 mRNA expression. However, IR combined with Ad5CMV-p53 infection stimulated significant increase in p53 mRNA expression supplemented with Bax and p21 mRNA expressions. In PC-3 (p53-null), IR induced Bax and p21 mRNA expression, while the combination effects were observed in p53, Bax, and p21 mRNA expression. Apoptotic cell deaths were rarely observed after IR alone (DU145: 3%, PC-3: 5%). However, after combination therapy, the proportion of apoptotic cells greatly increased (sevenfold in DU145 cells, and twice in PC-3 cells). G1 cell cycle arrest was observed after Ad5CMV-p53 infection and the combination in both cell lines. Conclusion: In this study, we demonstrated that the combination of IR and Ad5CMV-p53 gene therapy resulted in remarkable synergistic effects in human prostate cancer cells. This combination therapy could be one of the optimal treatment strategies for radioresistant prostate cancer.

http://abstracts.iovs.org/cgi/content/abstract/45/5/686

Purpose: The purpose was two-fold: 1) to develop economical quantitative PCR (real time) assays for mouse retinal genes expression that include matched primers/reference cDNA sets, and 2) to produce a companion database with practical information on reaction products and conditions. Methods: Several hot start Taq polymerases were compared for robustness with different primer sets. The gene expression Q-PCR kit development consisted of three parts: 1) computed evaluation of primer sets for intron/exon boundaries, literature errors, or custom design, 2) cloning of custom primers matched template cDNAs into pGemT Easy vector for use as standards, controls, and probes, and 3) experimental evaluation of annealing temperature ranges for each assay. Results: We have developed a collection of Q-PCR assays with companion standards and database for several retina specific genes. Those assays that have been developed are rhodopsin, PDE subunits alpha, beta, and gamma, blue opsin, green opsin, beta-actin, GAP-DH, Flt3, Fiz1, and NRL. We have found that custom assay conditions based on AmpliTaq Gold offers an economical advantage over pre-made kits. Conclusions: Q-PCR primer sets with matching standard cDNA were developed for measuring the expression of several retinal genes for use in the Eye Research Institute and will soon be available to the eye research community.


http://www.iovs.org/cgi/content/abstract/44/4/1657

PURPOSE. To describe the phenotype of an autosomal dominant macular dystrophy and identify the chromosomal locus. METHODS. Eleven members of a five-generation, nonconsanguineous British family were examined clinically and also underwent automated perimetry, electrodiagnostic testing, fundus fluorescein angiography, and fundus autofluorescence imaging. Blood samples were taken for DNA extraction and linkage analysis was performed. RESULTS. The phenotype is characterized by bull's-eye macular dystrophy first evident in the first or second decade of life. There is mild visual impairment, central scotomata, and electrophysiological testing indicates that most affected individuals have disease confined to the central retina but older subjects have more widespread rod and cone abnormalities, demonstrated by flash ERG. Genetic linkage analysis established linkage to chromosome 4 at p15.2-16.3 with a maximum lod score of 3.03 at a recombination fraction of 0.00 for marker D4S391. The locus for this autosomal dominant macular dystrophy lies between flanking markers D4S3023 and D4S3022, and overlaps the Stargardt 4 locus. CONCLUSIONS. A new locus was identified for a bull's-eye macular dystrophy on the short arm of chromosome 4.


http://www.iovs.org/cgi/content/abstract/44/10/4347

PURPOSE. To systematically explore changes in gene expression in the retina of monkeys with laser-induced glaucoma and to validate the microarray data on eyes with experimental glaucoma. METHODS. Glaucoma was induced in the right eye of four monkeys by repeated argon laser
photocoagulation of the trabecular meshwork. The left eye served as the control. Retinas were isolated from glaucomatous and control eyes 30 days after photocoagulation. Gene expression changes were analyzed by human microarray chips which displayed a total of 9182 elements including Expression Sequence Tag (EST) clones. Changes in the expression of some genes were further confirmed by real-time PCR analysis. Immunohistochemical studies to examine protein expression of some gene products were also done for several genes that showed up- or downregulation by the microarray analysis. RESULTS. Two eyes with mild glaucoma and two with severe glaucoma were produced. In the mild and severe glaucomatous retina, the number of upregulated genes was 45 and 18, and the number of downregulated genes was 17 and 21, respectively. The number of genes that were up- or downregulated was 0.7% of all the genes examined. The real-time PCR analysis confirmed expression changes of some genes found in the microarray analysis. Ceruloplasmin was one of the upregulated genes, and it was found by immunohistochemical analyses to be expressed in Muller cells. CONCLUSIONS. Gene expression profiles in laser-induced glaucomatous monkey retinas were determined, and only a very small population of genes was up- or downregulated in glaucomatous eyes. Upregulation of ceruloplasmin protein was found in the Muller cells.


http://www.iovs.org/cgi/content/abstract/43/6/1870

PURPOSE. The Emory mouse is a well-characterized model for age-onset cataract. The purpose of the present study was to identify differentially expressed genes between pre- and postcataract Emory mouse lenses. METHODS. Eyes were extracted from Emory mice at 3 weeks (precataract) and 7.5 months (postcataract) of age, and lenses were dissected. Lens RNA was compared for gene expression differences by RT-PCR differential display, and transcripts exhibiting altered levels of gene expression were cloned and identified by sequencing. The levels of two transcripts were further evaluated by RT-PCR in 3-week- and 7.5-month-old lenses and the remainder of the eye. The same transcripts were also measured in lenses from three non-Emory mouse strains (FVB/N, 129Sv, and CD1) ages 4 weeks to 11.5 months. RESULTS. Three transcripts were identified as exhibiting altered levels of gene expression between 3-week- and 7.5-month-old Emory mouse lenses. These encoded {alpha}A-crystallin (decreased), {beta}A3/A1-crystallin (decreased), and adhesion-related kinase (ARK) receptor tyrosine kinase (increased). Decreased {alpha}A-crystallin and increased ARK expression were not detected in lenses isolated from three non-Emory mouse strains of similar age. Increased expression of ARK was not detected between 3-week- and 7.5-month-old Emory mouse eye nonlens tissues. CONCLUSIONS. The present data confirm that expression of the {alpha}A-crystallin gene is decreased in cataract in the Emory mouse lens relative to age-matched control lenses and they provide evidence for cataract- and lens-specific upregulation of the ARK receptor tyrosine kinase in the Emory mouse.


http://abstracts.iovs.org/cgi/content/abstract/45/5/1071

Purpose: Primary intraocular lymphoma (PIOL) is a diffuse large B cell lymphoma (DLBCL) in which malignant lymphoid cells invade the retina, vitreous, or optic nerve head, with or without concomitant central nervous system involvement. The bcl-2 t(14;18) translocation brings the bcl-2 gene, an anti-apoptosis gene found on chromosome 18, under the control of the IgH promoter, located on chromosome 14, leading to Bcl-2 expression. This translocation is found in 85% of
follicular non-Hodgkin's lymphomas (FL) and 28% of diffuse large B-cell lymphomas (DLBCL). Sixty percent of Bcl-2 breakpoints are located at the major breakpoint region (Mbr) in the 3' non-coding part of the third exon. The next most frequent location for translocations (10-25%) is the minor cluster region (mcr) located 20 kb downstream of the gene. The purpose of this study was to examine the distribution of these bcl-2 breakpoints in PIOL. Methods: Polymerase chain reaction (PCR) was performed on DNA extracted from microdissected PIOL cells. The Mbr was analyzed in 69 patients, and the mcr was analyzed in 67 patients. The PCR-amplifiable mixture contained microdissected DNA, 4 pmol 32P-labeled sense primer of Mbr, 5'-TTAGAGACGTGGCTCTTAC-3' or mcr, 5'-GACTCTTTACGCTGGTACC-3', 5 pmol antisense primer (CFW1), 5'-ACCTTTAGGTGACCAGGGT-3', 10 nmol dNTP, 5 nmol MgCl2, and 0.5 U AmpliTaq Gold Enzyme in a final volume of 10 (micro)L. The PCR reaction for the Mbr was performed as follows: 94{degrees}C for 9 min, then 40 cycles of 94{degrees}C x 45 sec, 55{degrees}C x 45 sec, and 72{degrees}C x 1 min, followed by 72{degrees}C for 7 min. The PCR reaction for the mcr was performed as follows: 94{degrees}C for 9 min, then 35 cycles of 94{degrees}C x 1 min, 55{degrees}C x 2 min, and 72{degrees}C x 2 min, followed by 72{degrees}C for 7 min. The amplified DNA was separated on a 3% agarose gel. The gel was then stained with ethidium bromide and autoradiographed. Results: 37/69 (54%) PIOL patients expressed the t(14;18) translocation at the Mbr. 15/67 (22%) expressed the translocation at the mcr. Of these patients, 14/15 (93%) were also positive for the Mbr, indicating some overlap of the breakpoint regions, while 1/15 (7%) was positive only at the mcr. Conclusion: These results are similar to those seen in FL and DLBCL, indicating that this translocation may also play a role in PIOL pathogenesis. Furthermore, this translocation is used as a marker for diagnosis and monitoring of FL, although its role in prognosis and treatment selection for FL and DLBCL remains unclear. This study lays a foundation for future studies aimed at exploring the role of Bcl-2 expression in clinical presentation, treatment response, relapse, and survival in patients with PIOL.


http://abstracts.iovs.org/cgi/content/abstract/46/5/3465

Purpose: Primary intraocular lymphoma (PIOL) is typically a diffuse large B cell lymphoma of the retina, vitreous, or optic nerve head. The bcl-2 t(14;18) translocation brings the bcl-2 gene, an anti-apoptosis gene, under the control of the IgH promoter. This study examined the role of this translocation in PIOL survival and relapse. Methods: From 1991 to 2003, the NEI received ocular specimens from 72 patients with PIOL. Detailed clinical information was available for 23 patients. In order to detect the bcl-2 t(14;18) translocation at the Mbr, 15/67 (22%) expressed the translocation at the mcr. Of these patients, 14/15 (93%) were also positive for the Mbr, indicating some overlap of the breakpoint regions, while 1/15 (7%) was positive only at the mcr. Conclusion: These results are similar to those seen in FL and DLBCL, indicating that this translocation may also play a role in PIOL pathogenesis. Furthermore, this translocation is used as a marker for diagnosis and monitoring of FL, although its role in prognosis and treatment selection for FL and DLBCL remains unclear. This study lays a foundation for future studies aimed at exploring the role of Bcl-2 expression in clinical presentation, treatment response, relapse, and survival in patients with PIOL.
t(14;18) translocation. Conclusions: Studies have found conflicting roles for the bcl-2 t(14;18) translocation in systemic follicular and diffuse large B-cell lymphomas. Our study did not find a role for the bcl-2 t(14;18) translocation in determining length of survival or likelihood of disease relapse in PIOL, although it is interesting to note that patients with the bcl-2 t(14;18) translocation were significantly younger. This may suggest a role for the translocation in accelerating disease presentation and progression.

J Androl (1)


http://www.andrologyjournal.org/cgi/content/abstract/25/5/692

The role of tubular structures that contribute to the passage of spermatozoa is not solely passive; these structures actively contribute to their own functions, although these tubules and ducts are contiguous and collaborate in the development of the male gamete along their lengths. The testis has the specific function to generate spermatozoa and spermatozoa undergo numerous changes as they pass through the epididymis. A member of the p53 family of genes, p63, is highly expressed in the basal layers of epithelial tissues and plays a key role in maintaining their cell populations, whereas Notch 1 and its ligand Jagged 2 have an important role in the differentiation of germ cells and Jagged 2 is up-regulated by TAp63, one of the p63 isoforms, which transactivates p53 target genes and induces apoptosis. Although the presence of p63 in most epithelia is established, the role of p63 and its possible relationship with the Notch system in the seminiferous epithelium have not been examined. Therefore, we investigated the expression of p63, Jagged 2, and Notch 1 in the testis during postnatal development in comparison with their expression levels in the vaso-epididymal epithelium. In the testis, the expression of TAp63 mRNA increased at day 14 after birth and the expressions of Jagged 2 and Notch 1 mRNA increased at day 16 after birth, suggesting that TAp63-mediated Jagged 2 induction activates the Notch signaling system. On the other hand, the strong signal of (Delta)Np63 mRNA was already recognized in the vas deferens at day 0 after birth and advanced chronologically along the duct to the caput epididymis and p63 protein was expressed in basal cells in their epithelium, whereas the mRNAs of Jagged 2 and Notch 1 were maintained at a low level. Consequently, examination of our data raises the probability that TAp63 has an important role for maintenance of germ cell numbers, triggering or balancing the development, differentiation, and apoptosis of germ cells in the testis, which is completely different from the role of (Delta)Np63 in other epithelial tissues.

J Anim Sci (8)

An experiment was conducted to determine whether pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed cows could be improved by 1) culturing embryos in the presence of IGF-I and 2) treating recipients with GnRH. Lactating Holstein cows (n = 260) were synchronized using a timed ovulation protocol. Embryos were produced in vitro and cultured with or without 100 ng/mL of IGF-I. On d 7 after anticipated ovulation (d 0), a single embryo was transferred to all recipients with a palpable corpus luteum (n = 210). A subset of recipients (n = 164) was injected with either GnRH or placebo on d 11. Plasma progesterone concentrations on d 0 and 7 were used to determine the synchrony of recipients. Pregnancy was diagnosed at d 53 and 81 by rectal palpation. Among all recipients, transfer of IGF-I-treated embryos increased pregnancy rate at d 53 (P < 0.05) and tended to increase pregnancy rate at d 81 (P < 0.06). Calving rate also tended to be higher for recipients that received IGF-I-treated embryos (P < 0.07). Among the subset of synchronized recipients (n = 190), pregnancy rate at d 53 and d 81 and calving rate were higher (P < 0.05) for IGF-I-treated embryos. The GnRH tended to increase pregnancy rate at d 53 for all recipients (P < 0.08) and the subset of synchronized recipients (P < 0.10). There were no effects of GnRH (P > 0.10) for pregnancy rate at d 81 and calving rate. The overall proportion of male calves was 64.3%. There was no effect (P > 0.10) of embryo treatment or GnRH on the birth weight or sex ratio of calves. Results of this experiment indicate that treatment of embryos with IGF-I can improve pregnancy and calving rates following transfer of in vitro-produced embryos. Further research is necessary to determine whether the treatment of recipients with GnRH is a practical approach to increase pregnancy rates following in vitro embryo transfer.


Genetically modified corn has been approved as an animal feed in several countries, but information about the fate of genetically modified DNA and protein in vivo is insufficient. Genetically modified corn Bt11 is developed by inserting a recombinant DNA sequence encoding insecticidal Cry1Ab protein from Bacillus thuringiensis subsp. kurstaki. We examined the presence of corn intrinsic and recombinant cry1Ab gene by PCR, and the Cry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified corn Bt11-fed and five nongenetically modified corn-fed pigs. Fragments of corn zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified corn-fed pigs. Fragments of recombinant cry1Ab gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither corn intrinsic nor cry1Ab gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immunochromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested corn DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

Suggestive QTL affecting raw firmness scores and average Instron force, tenderness, juiciness, and chewiness on cooked meat were mapped to pig chromosome 2 using a three-generation intercross between Berkshire and Yorkshire pigs. Based on its function and location, the calpastatin (CAST) gene was considered to be a good candidate for the observed effects. Several missense and silent mutations were identified in CAST and haplotypes covering most of the coding region were constructed and used for association analyses with meat quality traits. Results demonstrated that one CAST haplotype was significantly associated with lower Instron force and cooking loss and higher juiciness and, therefore, this haplotype is associated with higher eating quality. Some of the sequence variation identified may be associated with differences in phosphorylation of CAST by adenosine cyclic 3', 5'-monophosphate-dependent protein kinase and may in turn explain the meat quality phenotypic differences. The beneficial haplotype was present in all the commercial breeds tested and may provide significant improvements for the pig industry and consumers because it can be used in marker-assisted selection to produce naturally tender and juicy pork without additional processing steps.


The ability to assess fertility of bovine sperm accurately and rapidly would be very useful for research and applications to the cattle industry. Sperm motility and other in vitro tests of sperm normality are only partially correlated with fertility, and lengthy breeding trials are expensive and time consuming. Heterospermic insemination by mixing sperm from more than one male provides an in vivo method to assess relative fertility among bulls that can be economical and rapid. Sperm that had been flow-sorted and cryopreserved from four groups of four bulls were inseminated in all combinations of three bulls within groups into nonsuperovulated heifers or superovulated heifers. Embryos were collected nonsurgically between d 13.5 and 20 following estrus and evaluated for paternity by genotyping. Following determination of paternity, a heterospermic index was created for each bull using a maximum likelihood function. These indices ranged from 0.22 (+/-) 0.15 to 2.43 (+/-) 0.43 (mean = 1.00, with a higher value indicative of greater fertility). In all four groups, either the high- or low-fertility bull was identified (P < 0.05) using a total of 25 to 36 genotypable embryos from nonsuperovulated heifers. The heterospermic rankings of bulls were similar for single and superovulated heifers for one group of bulls, but dissimilar for a second group. Heterospermic insemination followed by genotyping of embryos proved to be efficacious for rapidly ranking fertility of flow-sorted sperm from bulls when females were not superovulated, but results were less clear when females were superovulated.


Multiple nucleotide sequences of complementary DNA (cDNA) of bovine troponin T (TnT) isoforms expressed in the adult skeletal muscles were determined to facilitate the elucidation of the TnT degradation progress during postmortem aging of muscles. Fresh muscle samples were excised from the lingual, masseter, pectoralis, diaphragm, psoas major, longissimus thoracis, spinnalis, semitendinosus, semimembranosus, and biceps femoris muscles of three Holstein cows within 1 h of slaughter. Complementary DNA fragments of fast and slow TnT isoforms
expressed in each muscle were amplified by reverse-transcribed PCR. Consequently, four major fragments of fast TnT and two fragments of slow TnT, all of which contained the complete coding region, were obtained. The sequence determination of these fragments revealed that at least eight and two isoforms were generated by the alternative splicing from bovine fast and slow TnT messenger RNA, respectively. In the fast TnT isoforms, five small variable exons were observed; three of these five exons were in the amino (N)-terminal region. The calculated molecular weight of fast and slow TnT isoforms ranged from 29,816 to 32,125 and from 30,166 to 31,284, respectively. The deduced amino acid sequences revealed that the N-terminal region of all the TnT isoforms was extremely glutamic acid-rich. Reverse-transcribed PCR analysis revealed that expression of each of these isoforms was distributed in a fast or slow muscle-specific manner. Given that TnT degradation has been reported to accompany a decrease in glutamic acid content in the conventional 30-kDa degradation product, the sequence data suggested that the 30-kDa fragment seem to be generated by the proteolytic removal of the glutamic acid-rich N-terminal ends. The multiplicity of TnT isoforms may result in a complicated pattern of TnT degradation on SDS-PAGE gel during beef aging.


http://jas.fass.org


http://jas.fass.org/cgi/content/abstract/80/12/3077

Micromolar calcium activated neutral protease (CAPN1) was evaluated as a candidate gene for a quantitative trait locus (QTL) on BTA29 affecting meat tenderness by characterization of nucleotide sequence variation in the gene. Single-nucleotide polymorphisms (SNP) were identified by sequencing all 22 exons and 19 of the 21 introns in two sires (Piedmontese x Angus located at the U.S. Meat Animal Research Center in Clay Center, NE; Jersey x Limousin located at AgResearch in New Zealand) of independent resource populations previously shown to be segregating meat tenderness QTL on BTA29. The majority of the 38 SNP were found in introns or were synonymous substitutions in the coding regions, with two exceptions. Exons 14 and 9 contained SNP that were predicted to alter the protein sequence by the substitution of isoleucine for valine in Domain III of the protein, and alanine for glycine in Domain II of the protein. The resource populations were genotyped for these two SNP in addition to six intronic polymorphisms and two silent substitutions. Analysis of genotypes and shear force values in both populations revealed a difference between paternal CAPN1 alleles in which the allele encoding isoleucine at position 530 and glycine at position 316 associated with decreased meat tenderness (increased shear force values) relative to the allele encoding valine at position 530 and alanine at position 316 (P < 0.05). The association of maternal alleles with meat tenderness phenotypes is consistent with the hypothesis of CAPN1 as the gene underlying the QTL effect in two independent resource populations and presents the possibility of using these markers for selective breeding to reduce the numbers of animals with unfavorable meat tenderness traits.


Hypercholesterolemia (HC) is a major risk factor for the development of coronary heart disease. Coronary ion regulation, especially calcium, is thought to be important in coronary heart disease development; however, the influence of high dietary fat and cholesterol on coronary arterial smooth muscle (CASM) ion channels is unknown. The purpose of this study was to determine the effect of diet-induced HC on CASM voltage-gated calcium current (ICa). Male miniature swine were fed a high-fat, high-cholesterol diet (40% kcal fat, 2% wt cholesterol) for 20-24 wk, resulting in elevated serum total and low-density lipoprotein cholesterol. Histochemistry indicated early atherosclerosis in large coronary arteries. CASM were isolated from the right coronary artery (>1.0 mm ID), small arteries (≤200 μm), and large arterioles (≤100 μm). ICa was determined by whole cell voltage clamp. L-type ICa was reduced ~30% by HC compared with controls in the right coronary artery (-5.29 ± 0.42 vs. -7.59 ± 0.41 pA/pF) but not the microcirculation (small artery, -8.39 ± 0.80 vs. -10.13 ± 0.60; arterioles, -10.78 ± 0.93 vs. -11.31 ± 0.95 pA/pF). Voltage-dependent activation was unaffected by HC in both the macro- and microcirculation. L-type voltage-gated calcium channel (Cav1.2) mRNA and membrane protein levels were unaffected by HC. Inhibition of ICa by HC was reversed in vitro by the cholesterol scavenger methyl-β-cyclodextrin and mimicked in control CASM by incubation with the cholesterol donor cholesterol:methyl-β-cyclodextrin. These data indicate that CASM L-type ICa is decreased in large coronary arteries in early stages of atherosclerosis, whereas ICa in the microcirculation is unaffected. The inhibition of calcium channel activity in CASM of large coronary arteries is likely due to increases in membrane free cholesterol.


Recent studies have demonstrated that oxygen-sensitive type I cells in the carotid body express the gap junction-forming protein connexin43 (Cx43). In the present study, we examined the hypothesis that chronic exposure to hypoxia increases Cx43 expression in type I cells as well as in chemoafferent neurons in the petrosal ganglion. Immunocytochemical studies in tissues from normal rats revealed diffuse and granular Cx43-like immunoreactivity in the cytoplasm of type I cells and dense punctate spots of immunoreactive product at the margins of type I cells and near the borders of chemosensory cell lobules. Cx43-like immunoreactivity was not detectable in petrosal ganglion neurons from normal animals. After a 2-wk exposure to hypobaric (380 Torr) hypoxia, Cx43 immunostaining was substantially enhanced in and around type I cells. Moreover, chronic hypoxia elicited the expression of Cx43-like immunoreactivity in the cytoplasm of afferent neurons throughout the petrosal ganglion. Quantitative RT-PCR studies indicate that chronic hypoxia evokes a substantial increase in Cx43 mRNA levels in the carotid body, along with a
marked elevation of Cx43 expression in the petrosal ganglion. Increased Cx43 expression and gap junction formation in type I cells and sensory neurons may contribute to carotid body adaptation during sustained stimulation in extreme physiological conditions.


http://jap.physiology.org/cgi/content/abstract/97/5/1814

We hypothesized that adenovirus-mediated inducible nitric oxide synthase (iNOS) gene transduction of the lung would result in time-dependent iNOS overexpression and attenuate the vascular constrictor responses to a thromboxane mimetic, U-46619. Rats were treated via the trachea with surfactant alone (sham), surfactant containing an adenoviral construct with a cytomegalovirus promoter-regulated human iNOS gene (Adeno-iNOS), or an adenoviral construct without a gene insert (Adeno-Control). Adeno-iNOS-transduced rats demonstrated human iNOS mRNA and increased iNOS protein levels only in the lungs. Immunohistochemistry of lungs from Adeno-iNOS-treated animals demonstrated transgene expression in alveolar wall cells. In the lungs from Adeno-iNOS-transduced rats, the expression of iNOS protein and exhaled nitric oxide concentrations were increased on days 1-4 and 7 but returned to baseline values by day 14. The administration of the selective iNOS inhibitor L-N6-(1-iminoethyl)lysine dihydrochloride (L-NIL) decreased exhaled nitric oxide concentrations to levels found in Adeno-Control-transduced lungs. In a second group of rats, the segmental vasoconstrictor responses to U-46619 were determined in isolated, perfused lungs 3 days after transduction. Lungs from rats transduced with Adeno-iNOS had reduced total, arterial, and venous vasoconstrictor responses to U-46619 compared with sham, Adeno-Control, and control groups. In a third set of experiments, the response to 400 nM U-46619 in the presence of 10 μM L-NIL was not different in the isolated lungs from Adeno-Control- and Adeno-iNOS-transduced rats. We conclude that adenovirus-mediated iNOS gene transduction of the lung results in time-dependent iNOS overexpression, which attenuates the vascular constrictor responses to the thromboxane mimetic U-46619.


http://jap.physiology.org/cgi/content/abstract/92/6/2600

Prior induction of heat shock protein 70 (HSP70) protects against ischemia-reperfusion (I/R) mucosal injury, but the ability of HSP70 to affect I/R-induced alterations in epithelial cell function is unknown. Rats subjected to whole body hyperthermia (41.5-42(degrees)C for 6 min) increased HSP70 and heat shock factor 1 mRNA expression, reaching a maximum 2 h after heat stress and declining thereafter. HSP70 production was maximally elevated at 4 h after heat stress and remained elevated until after 12 h. Heat stress alone had no effect on mucosal function except to enhance secretion in response to ACh. Heat stress provided complete morphological protection against I/R-induced mucosal injury but did not confer a similar protection against I/R-induced decreases in mucosal resistance, sodium-linked glucose absorption, or tachykinin-mediated chloride secretion. Heat stress, however, attenuated the I/R-induced suppression of ACh response, and this effect was dependent on enteric nerves. Thus induction of heat shock protein 70 is associated with the preservation of mucosal architecture and attenuation of some specific functional alterations induced by I/R.
5'-AMP-activated protein kinase (AMPK) has been proposed to be a pivotal factor in cellular responses to both acute exercise and exercise training. To investigate whether protein levels and gene expression of catalytic ([alpha]1, [alpha]2) and regulatory ([beta]1, [beta]2, [gamma]1, [gamma]2, [gamma]3) AMPK subunits and exercise-induced AMPK activity are influenced by exercise training status, muscle biopsies were obtained from seven endurance exercise-trained and seven sedentary young healthy men. The [alpha]1- and [alpha]2-AMPK mRNA contents in trained subjects were both 117 +/- 2% of that in sedentary subjects (not significant), whereas mRNA for [gamma]3 was 61 +/- 1% of that in sedentary subjects (not significant). The level of [alpha]1-AMPK protein in trained subjects was 185 +/- 34% of that in sedentary subjects (P < 0.05), whereas the levels of the remaining subunits ([alpha]2, [beta]1, [beta]2, [gamma]1, [gamma]2, [gamma]3) were similar in trained and sedentary subjects. At the end of 20 min of cycle exercise at 80% of peak O2 uptake, the increase in phosphorylation of [alpha]-AMPK (Thr172) was blunted in the trained group (138 +/- 38% above rest) compared with the sedentary group (353 +/- 63% above rest) (P < 0.05). Acetyl CoA-carboxylase [beta]-phosphorylation (Ser221), which is a marker for in vivo AMPK activity, was increased by exercise in both groups but to a lower level in trained subjects (32 +/- 5 arbitrary units) than in sedentary controls (45 +/- 1 arbitrary units) (P < 0.01). In conclusion, trained human skeletal muscle has increased [alpha]1-AMPK protein levels and blunted AMPK activation during exercise.


http://jap.physiology.org/cgi/content/abstract/96/4/1292

Thirty strength-trained subjects were randomized to carbohydrate (CHO) or placebo (Pla) groups and lifted weights for 2 h (10 exercises, 4 sets each, 10 repetitions, with 2- to 3-min rest intervals). Subjects received 10 ml{middle dot}kg^{-1}{middle dot}h^{-1} CHO (6%) or Pla beverages during the weight training bout. Blood, saliva, and vastus lateralis muscle biopsy samples were collected before and after exercise. Blood cell counts were determined, and plasma was analyzed for IL-6, IL-10, IL-1 receptor antagonist (IL-1ra), IL-8, and cortisol. Muscle was analyzed for glycogen content and relative gene expression of 13 cytokines (IL-1{alpha}, IL-1{beta}, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-{gamma}, TNF-{alpha}) by use of real-time quantitative RT-PCR. Significant but modest increases were measured for plasma IL-6, IL-10, IL-1ra, and IL-8, but the pattern of increase did not differ between CHO and Pla groups. The rate of decrease in muscle glycogen content did not differ between CHO and Pla (P = 0.463). Muscle cytokine mRNA was detected preexercise for IL-1{beta}, IL-6, IL-15, IL-8, and TNF-{alpha}, and of these, IL-1{beta}, IL-6, IL-8, and TNF-{alpha} were significantly increased after the 2-h weight training bout. The increase in mRNA (fold difference from preexercise) did not differ between CHO and Pla groups. In summary, CHO vs. Pla ingestion did not alter modest increases measured for plasma IL-6, IL-10, IL-1ra, and IL-8, and muscle gene expression for IL-1{beta}, IL-6, IL-8, and TNF-{alpha} in strength-trained subjects lifting weights intensively for 2 h.


http://jap.physiology.org/cgi/content/abstract/94/5/1917
Sixteen experienced marathoners ran on treadmills for 3 h at ~70% maximal oxygen consumption ([V]O2 max) on two occasions while receiving 1 l/h carbohydrate (CHO) or placebo (Pla) beverages. Blood and vastus lateralis muscle biopsy samples were collected before and after exercise. Plasma was analyzed for IL-6, IL-10, IL-1 receptor agonist (IL-1ra), IL-8, cortisol, glucose, and insulin. Muscle was analyzed for glycogen content and relative gene expression of 13 cytokines by using real-time quantitative RT-PCR. Plasma glucose and insulin were higher, and cortisol, IL-6, IL-10, and IL-1ra, but not IL-8, were significantly lower postexercise in CHO vs. Pla. Change in muscle glycogen content did not differ between CHO and Pla (P = 0.246). Muscle cytokine mRNA content was detected preexercise for seven cytokines in this order (highest to lowest): IL-15, TNF-[alpha], IL-8, IL-1[beta], IL-12p35, IL-6, and IFN-[gamma]. After subjects ran for 3 h, gene expression above prerun levels was measured for five of these cytokines: IL-1[beta], IL-6, and IL-8 (large increases), and IL-10 and TNF-[alpha] (small increases). The increase in mRNA (fold difference from preexercise) was attenuated in CHO (15.9-fold) compared with Pla (35.2-fold) for IL-6 (P = 0.071) and IL-8 (CHO, 7.8-fold; Pla, 23.3-fold; P = 0.063). CHO compared with Pla beverage ingestion attenuates the increase in plasma IL-6, IL-10, and IL-1ra and gene expression for IL-6 and IL-8 in athletes running 3 h at 70% [V]O2 max despite no differences in muscle glycogen content.


http://jap.physiology.org/cgi/content/abstract/95/3/1201

Changes in gene expression during recovery from high-intensity, intermittent, one-legged exercise were studied before and after 5.5 wk of training. Genes related to metabolism, as well as Na+, K+, and pH homeostasis, were selected for analyses. After the same work was performed before and after the training period, several muscle biopsies were obtained from vastus lateralis muscle. In the untrained state, the Na+-K+-ATPase [alpha]1-subunit mRNA level was approximately threefold higher (P < 0.01) at 0, 1, and 3 h after exercise, relative to the preexercise resting level. After 3-5 h of recovery in the untrained state, pyruvate dehydrogenase kinase 4 and hexokinase II mRNA levels were elevated 13-fold (P < 0.001) and 6-fold (P < 0.01), respectively. However, after the training period, only pyruvate dehydrogenase kinase 4 mRNA levels were elevated (P < 0.05) during the recovery period. No changes in resting mRNA levels were observed as a result of training. In conclusion, cellular adaptations to high-intensity exercise training may, in part, be induced by transcriptional regulation. After training, the transcriptional response to an exercise bout at a given workload is diminished.


http://jap.physiology.org/cgi/content/abstract/95/3/1038

Increasing evidence suggests that the myogenic regulatory factors (MRFs) and IGF-I have important roles in the hypertrophy response observed after mechanical loading. We, therefore, hypothesized that a bout of heavy-resistance training would affect the MRF and IGF-I mRNA levels in human skeletal muscle. Six male subjects completed four sets of 6-12 repetitions on a leg press and knee extensor machine separated by 3 min. Myogenin, MRF4, MyoD, IGF-I Eb (isoforms a, b, and c) and IGF-I Ebc (isofrom b and c) mRNA levels were determined in the vastus lateralis muscle by RT-PCR before exercise, immediately after, and 1, 2, 6, 24, and 48 h postexercise. Myogenin, MyoD, and MRF4 mRNA levels were elevated (P < 0.005) by 100-400% 0-24 h postexercise. IGF-I Ebc mRNA content decreased (P < 0.005) by [~]44% after 1 and 6 h of recovery. The IGF-I Eb mRNA level was unaffected. The present study shows that myogenin,
MyoD, and MRF4 mRNA levels are transiently elevated in human skeletal muscle after a single bout of heavy-resistance training, supporting the idea that the MRFs may be involved in regulating hypertrophy and/or fiber-type transitions. The results also suggest that IGF-I\textsuperscript{Ea} expression may be downregulated at the mRNA level during the initial part of recovery from resistance exercise.


http://jap.physiology.org/cgi/content/abstract/95/1/104

Androgens have been implicated as the causative factor for the postinjury immune dysfunction in males; however, it remains unknown whether androgens directly affect macrophages. To study this, male mice were sham operated or subjected to trauma (i.e., midline laparotomy) and hemorrhagic shock (mean arterial pressure, 30 \(+/-\) 5 mmHg for 90 min and then resuscitated). The mice received the 5\textit{alpha}-reductase inhibitor 4-hydroxyandrostenedione (4-OHA) before resuscitation. Plasma TNF-{\alpha}, IL-6, and IL-10 levels were elevated after trauma-hemorrhage and normalized by 4-OHA. TNF-{\alpha} and IL-6 production by splenic macrophages was decreased after injury, whereas Kupffer cell production of these mediators was enhanced. 4-OHA normalized cytokine production. Androgens suppressed cytokine production by splenic macrophages from hemorrhaged mice, whereas it enhanced TNF-{\alpha} and IL-6 production by Kupffer cells. The addition of 4-OHA in vitro normalized cytokine production by cells treated with testosterone, but it had no effect on dihydrotestosterone-treated cells. These results indicate that androgens directly affect macrophage function in males after trauma and hemorrhagic shock and that the intracellular conversion of testosterone to dihydrotestosterone is of particular importance in mediating the androgen-induced effects.


http://jap.physiology.org/cgi/content/abstract/92/3/1064

The purpose of this study was to identify genetic targets in the vasculature for estrogen by profiling genes expressed in female human aortic endothelial cells exposed to various doses of 17\textit{beta}-estradiol at differing concentrations and for differing periods of time. Our approach employed a RT-PCR-based cloning strategy of DNA differential display analysis, with differential expression verified by semiquantitative PCR performed with gene-specific primers. A significant increase in mRNA expression in response to 17\textit{beta}-estradiol was observed for the following three genes: aldose reductase (3.4-fold), caspase homologue-{\alpha} protein (4.2-fold), and plasminogen activator inhibitor-1 intron e (2.3-fold). For all three upregulated genes, estradiol-induced upregulation occurred with a similar time course and temporally clustered to the first 24 h after hormone treatment. In addition, the effect of estradiol dose on gene expression was consistent and occurred at physiological concentrations. Our results describe previously uncharacterized estradiol-sensitive time- and dose-dependent regulation of genes with potential importance to vascular function in human endothelial cells.
The hypothalamic suprachiasmatic nuclei (SCN), the principal circadian oscillator in mammals, are synchronized to the solar day by the light-dark cycle, and in turn, they coordinate circadian oscillations in peripheral tissues. The tau mutation in the Syrian hamster is caused by a point mutation leading to a deficiency in the ability of Casein Kinase 1{epsilon} to phosphorylate its targets, including circadian PER proteins. How this accelerates circadian period in neural tissues is not known, nor is its impact on peripheral circadian oscillators established. We show that this mutation has no effect on per mRNA expression nor the nuclear accumulation of PER proteins in the SCN. It does, however, accelerate the clearance of PER proteins from the nucleus to an extent sufficient to explain the shortened circadian period of behavioral rhythms. The mutation also has novel, unanticipated consequences for circadian timing in the periphery, including tissue-specific phase advances and/or reduced amplitude of circadian gene expression. The results suggest that the tau mutation accelerates a specific phase, during mid-late subjective night of the SCN circadian feedback loop, rather than cause a global compression of the entire cycle. This reprogrammed output from the clock is associated with peripheral desynchrony, which in turn could account for impaired growth and metabolic efficiency of the mutant.


The circadian system is thought to have three components: input, pacemaker (internal clock), and output. Cryptochromes (Cry) are important clock genes, and recent findings indicate that these genes not only act as circadian photoreceptors but are also essential components in the negative feedback of the circadian system. As a first step toward understanding the avian circadian system, the authors tried to clone Japanese quail homologs of mammalian Crys and analyze their expression patterns in different circumstances. Partial cDNAs of qCry1 and qCry2, which are homologs of mammalian Cry1 and Cry2, respectively, were obtained and their gene expressions were analyzed. Both qCry1 and qCry2 mRNAs were present in all the tissues examined. The oscillation patterns of the qCry1 transcripts were tissue specific and generally showed robust changes between daytime and nighttime; except for lung and testis tissues (which showed no detectable changes between daytime and nighttime), daytime levels were higher in all of the tissues examined. This rapid oscillation in qCry1 persisted through constant darkness or constant illumination, indicating that an endogenous clock controls these changes. In contrast, the expression of qCry2 did not oscillate in any tissue examined. In addition, in tissues of the pineal gland and eye, unexpected light exposure in the dark period was able to block the decrease in qCry1 transcripts or induce its expression. These findings, in conjunction with the established roles of CRYs in other species, led the authors to propose that in the circadian system, qCRYs may play important roles similar to the known roles of CRYs of other species, such as acting as circadian photoreceptors and as components of the circadian system.

Aging alters numerous aspects of circadian biology, including the amplitude of rhythms generated by the suprachiasmatic nuclei (SCN) of the hypothalamus, the site of the central circadian pacemaker in mammals, and the response of the pacemaker to environmental stimuli such as light. Although previous studies have described molecular correlates of these behavioral changes, to date only 1 study in rats has attempted to determine if there are age-related changes in the expression of genes that comprise the circadian clock itself. We used in situ hybridization to examine the effects of age on the circadian pattern of expression of a subset of the genes that comprise the molecular machinery of the circadian clock in golden hamsters. Here we report that age alters the 24-h expression profile of Clock and its binding partner Bmal1 in the hamster SCN. There is no effect of age on the 24-h profile of either Per1 or Per2 when hamsters are housed in constant darkness. We also found that light pulses, which induce smaller phase shifts in old animals than in young, lead to decreased induction of Per1, but not of Per2, in the SCN of old hamsters.


Prokineticin 2 (PK2) is a putative output molecule from the SCN. PK2 RNA levels are rhythmic in the mouse SCN, with high levels during the day, and PK2 administration suppresses nocturnal locomotor activity in rats. The authors examined the PK2 system in a diurnal rodent, Arvicanthis niloticus, to determine whether PK2 or PK2 receptors differ between diurnal and nocturnal species. The major transcript variant of A. niloticus PK2 (AnPK2) encodes a 26-residue signal peptide followed by the presumed mature peptide of 81 residues. Within the grass rat signal sequence, polymorphic sequences and amino acid substitutions were observed relative to mouse and laboratory rats, but the hydrophobic core and cleavage site of the signal sequence were preserved. The mature PK2 peptide is identical among A. niloticus, rat, and mouse. AnPK2 mRNA is rhythmically expressed in the SCN, with peak RNAlevels occurring in the morning, preceding peaks of Per1 and Per2 as in mouse SCN. Analysis of prokineticin receptor 2 (PKR2) sequences revealed polymorphisms among the grass rats studied. PKR2 mRNA was expressed in the SCN and paraventricular nuclei of the thalamus and hypothalamus. While further analysis is necessary, there is no clear evidence indicating that a difference in the PK2 ligand/receptor system accounts for diurnality in this rodent species. These data contribute to a growing body of evidence suggesting that the key to diurnality lies downstream of the SCN in A. niloticus.

Inositol phosphates (IPs), such as 1,4,5-inositol-trisphosphate (IP3), comprise a ubiquitous intracellular signaling cascade initiated in response to G protein-coupled receptor-mediated activation of phospholipase C. Classical methods for measuring intracellular accumulation of these molecules include time-consuming high-performance liquid chromatography (HPLC) separation or large-volume, gravity-fed anion-exchange column chromatography. More recent approaches, such as radio-receptor and AlphaScreen™ assays, offer higher throughput. However, these techniques rely on measurement of IP3 itself, rather than its accumulation with other downstream IPs, and often suffer from poor signal-to-noise ratios due to the transient nature of IP3. The authors have developed a miniaturized, anion-exchange chromatography method for measuring inositol phosphate accumulation in cells that takes advantage of signal amplification achieved through measuring IP3 and downstream IPs. This assay uses centrifugation of 96-well-formatted anion-exchange mini-columns for the isolation of radiolabeled inositol phosphates from cell extracts, followed by low-background dry-scintillation counting. This improved assay method measures receptor-mediated IP accumulation with signal-to-noise and pharmacological values comparable to the classical large-volume, column-based methods. Assay validation data for recombinant muscarinic receptor 1, galanin receptor 2, and rat astrocyte metabotropic glutamate receptor 5 are presented. This miniaturized protocol reduces reagent usage and assay time as compared to large-column methods and is compatible with standard 96-well scintillation counters.


http://jbx.sagepub.com/cgi/content/abstract/9/7/625

The human LMNA gene, when mutated, has been shown to cause at least 7 human diseases: dilated cardiomyopathy, Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, familial partial lipodystrophy, Charcot Marie tooth disease type II, mandibuloacral dysplasia, and Hutchinson-Gilford Progeria (OMIM #176670). This article describes a high-throughput method for screening the human lamin A/C (LMNA) gene for genetic mutations and sequence variation using denaturing high-performance liquid chromatography (DHPLC). In the present study, 76 patients with dilated cardiomyopathy were screened for mutations using DHPLC and sequence analysis. Abnormal elution profiles were identified and sequenced on an ABI 377 automatic sequencer. Heterozygous LMNA mutations were detected in 8% of the affected patients. In addition, a number of intronic and exonic single nucleotide polymorphisms were identified. LMNA mutations are clinically relevant in at least 6 human diseases. This study provides a protocol for high-throughput LMNA analysis applicable both in the research and in the clinical diagnostic setting.


http://jbx.sagepub.com/cgi/content/abstract/9/8/704

With the sequence of the human genome at hand, target discovery strategies are needed that can rapidly identify novel gene products involved in human disease pathways. In this article, the authors describe a cell-based, high-throughput assay that can identify gene products capable of modulating the vascular endothelial growth factor (VEGF) and tumor necrosis factor * (TNFa) signaling pathways in human endothelial cells. The assay uses real-time PCRtechnology to measure downstream reporter mRNA transcripts induced upon cytokine stimulation in a 96-well plate format and has been adapted for use with recombinant adenoviruses. The authors
specifically demonstratemodulation of cytokine-driven reporter transcripts using drug inhibitors and through adenoviral-mediated expression of known signaling intermediates of the respective pathways. In addition, they have used an arrayed library of 350 recombinant adenoviruses to screen for novel modulators of the VEGF and TNF* pathways. The high-throughput screening capacity and sensitivity of this system make it a useful tool for new drug target identification.


http://jds.fass.org/cgi/content/abstract/87/12/4104

Bovine subclinical mastitis can be defined as a moderated inflammatory disease characterized by a persistent accumulation of neutrophils in milk. As GMCSF-mediated delay of neutrophil apoptosis contributes to the accumulation of inflammatory cells at the site of inflammation in many human diseases, we sought to determine whether subclinical mastitis in cows is also associated with a GMCSF-dependent increase in milk-neutrophil survival. We first addressed the hypothesis that GMCSF delays bovine neutrophil apoptosis by activation of the signal transducer and activator of transcription (STAT) family members STAT3 and STAT5, which are critical regulators of the expression of various Bcl-2 family proteins. Granulocyte-macrophage colony-stimulating factor significantly delayed apoptosis of blood neutrophils obtained from healthy cows. In these cells, GMCSF activated STAT5, but not STAT3, and induced an increase in the mRNA of the antiapoptotic Bcl-2 member, Bcl-xL. Granulocyte-macrophage colony-stimulating factor-dependent STAT5 activation and up-regulation of Bcl-xL mRNA were blocked by the Jak inhibitor, AG-490. This inhibition was associated with abrogation of the prosurvival effect of GMCSF, demonstrating a key role for STAT5 in delayed neutrophil apoptosis. We further found that GMCSF expression was increased in milk cells from cows affected with subclinical mastitis. Neutrophils from these cows demonstrated a significant delay of apoptosis as compared with neutrophils obtained from healthy cows and were unresponsive to GMCSF. Active STAT5 complexes were detected in these neutrophils. Finally, in the presence of AG-490, apoptosis was induced and a time-dependent down-regulation of Bcl-xL mRNA was observed in milk neutrophils from mastitis-affected cows. These results indicate that neutrophil survival is enhanced in milk of subclinical mastitis-affected cows and suggest a role for a GMCSF-activated STAT5 signaling pathway in this phenomenon. This pathway could thus represent a target for the control of persistent accumulation of neutrophils in the bovine mammary gland.


http://jds.fass.org/cgi/content/abstract/86/4/1429

Stems of orchardgrass hay in nylon bags were incubated in the rumens of three ruminally fistulated sheep to monitor the rate and extent of fiber attachment by the representative ruminal cellulolytic bacteria via competitive polymerase chain reaction. After incubation for 5 min, the numbers of Fibrobacter succinogenes and the two ruminococcal species attached to stems were 105 and 104/g dry matter (DM) of stem, respectively. At 10 min, the numbers of all three species
attached to stems increased 10-fold. Thereafter, attached cell numbers of the three species gradually increased and peaked at 24 h (109/g DM for F. succinogenes and 107/g DM for Ruminococcus flavefaciens) or 48 h (106/g DM for Ruminococcus albus). On the other hand, cell numbers of all three species in the whole digesta were constant over 24 h. Changes in the rate of in sacco neutral detergent fiber disappearance of hay stem, which showed a linear increase up to 96 h, were not synchronized with changes in cellulytic bacterial mass. These results suggest that sufficient numbers of cells of the three cellulytic species to move to new plant fragments are present at the start of incubation, the initial attachment to new plant matter is mostly accomplished within 10 min and then bacterial growth and fibrolytic action follow. F. succinogenes was most dominant, both in the whole rumen digesta and on the suspended hay stems, demonstrating the ecological and functional significance of this species in ruminal fiber digestion.

http://jds.fass.org/cgi/content/abstract/85/4/707

For some French Registered Designation of Origin (RDO) cheeses Prim'Holstein's milk is not allowed for cheese making (e.g., Reblochon, Abondance, and Beaufort cheeses). To find molecular markers for Prim'Holstein's milk detection in RDO cheese, four genes affecting coat color in cattle (c-kit, MGH, TYRP1, and MC1R) have been sequenced for three mountain breeds and the Prim'Holstein breed. Only the MC1R gene (E-locus) has shown variation between the four breeds. Among the 25 French and Italian breeds sequenced for the MC1R gene, only the Vosgienne breed has presented the same allele as the black Prim'-Holstein breed (ED). A quick and easy DNA-based method to detect Holstein's milk in RDO cheese is proposed based on ED allele detection. A DNA extraction from cheese, a preamplification of the gene and a competitive oligonucleotide priming PCR on MC1R mutations were performed. Using an automated sequencer, differences in fluorescence and fragment size reveal the allele type. This simple approach provides good reproducibility and is shown to be relatively sensitive, with a detection limit of about 1% of Holstein's milk in milk curd.

http://jds.fass.org/cgi/content/abstract/87/11/3770

The prevalence of Mycobacterium avium ssp. paratuberculosis (Mptb) in culled dairy cattle in Eastern Canada and Maine was determined to be 16.1% (95% confidence interval 13.8 to 18.3%) based on a systematic random sample of abattoir cattle. Mesenteric lymph nodes and ileum from 984 cows were examined by histologic and bacteriologic methods. Histological testing was far less sensitive than bacteriologic methods for detecting infected cattle. A seasonal pattern of positive cows was also detected, with the highest proportion of cows being Mptb-positive in June (42.5%). Overall, body condition score was not associated with prevalence of Mptb isolation.

http://jds.fass.org/cgi/content/abstract/86/8/2715
\(\text{\(\kappa\)}\text{-Casein (\(\kappa\)}\text{-CN) is the milk protein that determines the size and specific function of milk micelles, and its cleavage by chymosin is responsible for milk coagulation. We have previously detected and characterized four variants of the goat\(\kappa\)\text{-CN in Spanish, French, and Italian breeds by screening the major part of the coding region in exon 4. Here we have sequenced and analyzed the full coding region of the\(\kappa\)\text{-CN gene which includes exons 3 and 4. No additional mutations were found, with exception of a single nucleotide substitution in exon 3, which had no amino acid change. However, the analysis of the association between the different mutations resulted in two new variants designated\(\kappa\)\text{-CN F and G. The novel variants are present in the Italian breeds Teramana, Girgentana, and Sarda (variant F). A protocol for rapid simultaneous genotyping of all known\(\kappa\)\text{-CN variants using the primer extension method was described, and a total of 210 animals from nine European breeds were genotyped. Alleles A and B are the most frequent variants occurring in the majority of breeds with highest prevalence of the B variant, except for the Canaria breed where the A allele is more frequent. Sequence data suggest that the F variant is the original type of caprine\(\kappa\)\text{-CN, other alleles being derived from this type following two different trunks by successive mutations.}}\)}

\(\text{J Forensic Sci (2)}\)


STR multiplexes have been indispensable for the efficient genotyping of forensic samples. The PowerPlex 16 System contains the coreCODIS loci, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, THO1, TPOX, vWA, the sex determinant locus, amelogenin, and two pentanucleotide STR loci, Penta D and Penta E. This multiplex satisfies the locus requirements for most national databases and is the most efficient currently available system due to its single PCR amplification. To provide the groundwork for judicial acceptance, including the publication of primer sequences, and to evaluate laboratory-to-laboratory variation, a developmental validation for casework on this commercially available system was performed in 24 laboratories and produced the following conclusions. Amplification was reliable on a variety of thermal cyclers and product could be analyzed on either an ABI PRISM 310 Genetic Analyzer or an ABI PRISM 377 DNA Sequencer. Genotyping using single source samples was consistent between 0.25 and 2 ng of input DNA template with a few laboratories obtaining complete genotypes at 0.0625 ng. However, heterozygote allele imbalance (<60% peak height balance) caused by stochastic effects was observed at a rate of 13% with 0.125 ng DNA and 22% at 0.0625 ng DNA. Mixture analyses were done using a total of 1 ng of DNA template. Most alleles were detected in mixtures of 4 to 1 and some minor alleles were detected in mixtures of 19 to 1. Optimum amplification cycle number was dependent on the sensitivity of the detection instrument used and could also be adjusted to accommodate larger amounts of DNA on solid supports such as FTA paper. Reaction conditions including volume, annealing temperature, and concentrations of primer, AmpliTaq Gold, and magnesium were shown to be optimal yet robust enough to withstand moderate variations without affecting genotype analysis. Environmental, matrix and standard source analyses revealed an ability to obtain complete genotypes in all sample types except those exposed to 80 degrees C for 12-48 days. Finally, comparison of genotype results from the PowerPlex 16 System with other commercially available systems on non-probative reference and forensic samples showed consistent results.

Quality assurance samples submitted from the NCSBI as part of a contract with TBTG to outsource DNA Database samples showed unexpected discrepancies for the locus D16S539 when all other loci yielded identical results. Discrepancies observed included allele drop out and an imbalance in sister alleles with samples returned from TBTG. This led to a comprehensive review of the technical procedures used between the two laboratories to determine the cause of the discrepancies noted for the locus D16S539, since both laboratories were using the PowerPlex 1.1 typing kit from the Promega Corporation. The NCSBI and the TBTG utilize different extraction methods (organic extraction vs. FTA) and amplification conditions (AmpliTaq vs AmpliTaq Gold), respectively, so the exact cause of discrepancy observed was not immediately apparent. Experiments at the NCSBI associated the observed allele drop out and the imbalance of the sister alleles with the use of AmpliTaq Gold and a hot start procedure. Sequencing data revealed that a point mutation resides on the D16S539 primer-binding site that reaches polymorphic levels in African-American populations. This led to the development of a degenerate primer by the Promega Corporation to detect "missing" alleles when AmpliTaq Gold is used. The degenerate primer was then thoroughly tested to show its efficacy in detecting the "true" D16S539 profile when used.


Two modifications to the original L1 consensus primer human papillomavirus (HPV) PCR method, MY09-MY011, using AmpliTaq DNA polymerase (MY-Taq), were evaluated for HPV DNA detection on clinical specimens from a cohort study of cervical cancer in Costa Rica. First, HPV DNA testing of 2978 clinical specimens by MY09-MY011 primer set, using AmpliTaq Gold DNA polymerase (MY-Gold) were compared with MY-Taq testing. There was 86.8% total agreement (kappa = 0.72, 95%CI = 0.70-75) and 69.6% agreement among positives between MY-Gold and MY-Taq. MY-Gold detected 38% more HPV infections (P < 0.0001) and 45% more cancer-associated (high-risk) HPV types (P < 0.0001) than MY-Taq, including 12 of the 13 high-risk HPV types. Analyses of discordant results using cytologic diagnoses and detection of HPV DNA by the Hybrid Capture 2 Test suggested that MY-Gold preferentially detected DNA positive specimens with lower HPV viral loads compared with MY-Taq. In a separate analysis, PGMY09-PGMY11 (PGMY-Gold), a redesigned MY09/11 primer set, was compared with MY-Gold for HPV DNA detection (n = 439). There was very good agreement between the two methods (kappa = 0.83; 95%CI = 0.77-0.88) and surprisingly no significant differences in HPV detection (P = 0.41). In conclusion, we found MY-Gold to be a more sensitive assay for the detection of HPV DNA than MY-Taq. Our data also suggest that studies reporting HPV DNA detection by PCR need to report the type of polymerase used, as well as other assay specifics, and underscore the need for

http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/13/961

Background: The p53 gene is frequently mutated in non-small-cell lung cancer (NSCLC); however, the effect of p53 gene mutations on patient prognosis remains unclear. Therefore, we initiated a prospective study to determine the association of p53 gene mutations with survival in patients with stage I NSCLC. Methods: Tumor samples were collected prospectively from 188 patients with operable NSCLC (stages I, II, and IIIA). p53 mutations were detected by direct dideoxynucleotide sequencing and p53 GeneChip analysis. Association of clinical and pathologic variables (e.g., alcohol consumption, sex, age, pathologic stage) with mutation of the p53 gene was determined by logistic regression. Associations between p53 mutation status, clinical and pathologic variables, and survival were assessed using a Cox proportional hazards regression model. All statistical tests were two-sided. Results: p53 mutations were detected in 55% (104/188) of tumors. These mutations were associated with non-bronchoalveolar tumors, a history of alcohol consumption, and younger patient age. The risk of death was statistically significantly higher in patients with p53 mutations in their tumors (hazard ratio [HR] = 1.6, 95% confidence interval [CI] = 1.0 to 2.4; P = .049) than in patients with wild-type p53 in their tumors. Tumor stage, the presence of a p53 mutation, and increasing patient age were statistically significant predictors of patient death in the entire patient group; however, the statistically significant prognostic effect of p53 mutation was limited to patients with stage I NSCLC (stage I HR = 2.8, 95% CI = 1.4 to 5.6; stage II HR = 1.8, 95% CI = 0.74 to 4.4; and stage III HR = 0.70, 95% CI = 0.32 to 1.5). Among patients with stage I NSCLC, actuarial 4-year survival was statistically significantly higher in those with wild-type p53 than in those with mutant p53 (78% versus 52%, respectively; difference in 4-year survival = 26%, 95% CI = 6% to 46%; P = .009, log-rank test). Conclusion: Tumor p53 mutations are a statistically significant predictor of poor outcome in patients with stage I NSCLC.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/18/1394

Background: Human MYCN (hMYCN) oncogene amplification is a powerful predictor of treatment failure in childhood neuroblastoma, and dysregulation of hMYCN protein expression appears to be critically involved in the pathogenesis of this disease. We used hMYCN antisense (AS) oligonucleotides to investigate, both in vitro and in vivo, the therapeutic potential of inhibiting hMYCN expression. Methods: We transiently transfected human neuroblastoma IMR-32 cells, which have an amplified hMYCN gene, with fluorescently labeled hMYCN AS or scrambled (SCR) control oligonucleotides and used fluorescence-activated cell sorting to enrich for cell populations containing different levels of the oligonucleotides. We used fluorescence immunocytochemistry or reverse transcription polymerase chain reaction to assay gene expression levels and trypan blue
exclusion to assay growth inhibition in the cell populations. We examined the effects of continuous treatment for 6 weeks with AS or SCR oligonucleotides via subcutaneously implanted microosmotic pumps on tumor growth in a transgenic mouse model of hMYCN-induced neuroblastoma (n = 20 mice per group). All statistical tests were two-sided. Results: IMR-32 cells treated with AS oligonucleotides had approximately half as much hMYCN protein and cell proliferation as either SCR oligonucleotide-transfected or mock-transfected controls; the differences were statistically significant. Transgenic mice treated with AS oligonucleotides had lower tumor incidence and statistically significantly lower tumor mass than SCR-treated or untreated control mice. Compared with control treatments, AS oligonucleotide treatment in vitro and in vivo was associated with decreased expression of hMYCN and putative hMYCN target genes but not with that of closely related genes. Several AS oligonucleotide-treated mice developed tumors contralateral to the site of oligonucleotide administration, whereas SCR oligonucleotide-treated or untreated mice displayed bilateral tumor growth. Conclusions: Decreased expression of hMYCN protein is achievable with the use of AS oligonucleotide treatment, even in the presence of hMYCN oncogene amplification. Antisense strategies targeting the hMYCN oncogene in vivo decrease mouse neuroblastoma tumorigenesis. Investigation of their clinical effect in children with neuroblastoma is warranted.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;94/5/375

Background: Gastrin-releasing peptide receptor (GRPR)-mediated autocrine growth appears to be an early marker of susceptibility to tobacco-related lung cancers. Because expression of GRPR, however, has not been reported in squamous cell carcinoma of the head and neck (SCCHN), we investigated its expression and that of its ligand GRP in normal mucosa and SCCHN tissues and the involvement of these proteins in the proliferation of SCCHN cells. Methods: We assessed GRPR messenger RNA (mRNA) expression in specimens from 25 patients with SCCHN, six control noncancer patients, and 14 SCCHN cell lines by use of quantitative reverse transcriptase-polymerase chain reaction. We used neutralizing GRP monoclonal antibody 2A11 to block the GRP-GRPR interaction in SCCHN cell lines and xenografts and assessed the antibody's effect on proliferation by counting cultured cells or measuring xenograft tumor volume in vivo. All statistical tests were two-sided. Results: Tumor and mucosa tissues, respectively, from SCCHN patients expressed sixfold and fourfold higher levels of GRPR mRNA than normal mucosa tissue from noncancer patients (P<.001). The levels of GRPR expression in the tumor and adjacent normal epithelium of individual patients with SCCHN were correlated (r =.652; P =.001), suggesting that increased GRPR expression is an early event in SCCHN formation. SCCHN cells expressed fivefold higher levels of GRPR mRNA than did cultured normal mucosal epithelial cells (P =.005). GRP stimulated proliferation of SCCHN cells in a dose-dependent fashion (P =.006). Neutralizing GRP monoclonal antibody 2A11 inhibited SCCHN cell proliferation in vitro and in vivo. Median survival was 54 months in patients with higher levels of GRPR mRNA and was not reached in those with lower levels. Conclusions: GRP and GRPR appear to participate in an autocrine regulatory pathway in SCCHN. Thus, strategies that specifically target GRP and/or GRPR may be effective therapeutic approaches for this disease.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;96/1/15
Mutations in BRCA1 and/or BRCA2 (BRCA1/2) profoundly increase the risks of breast and ovarian cancers, but it is unclear whether mutations in these genes increase the risk of colorectal cancer. We investigated BRCA1/2 founder mutations and a family history of breast cancer as potential risk factors for colorectal cancer. Methods: In the population-based Molecular Epidemiology of Colorectal Cancer study in northern Israel, 1422 case patients with incident colorectal cancer, diagnosed between March 31, 1998, and December 31, 2002, and 1566 control subjects without colorectal cancer were genotyped for the BRCA1 187delAG, BRCA1 5385insC, and BRCA2 6174delT founder mutations. Genotypes and interview data from all case patients and control subjects and from only those of Ashkenazi Jewish descent (1002 case patients and 1038 control subjects) were used to calculate odds ratios [ORs] from logistic regression. Results: Twenty-four (2.4%) case patients and 20 (1.9%) control subjects carried one of the three mutations (OR = 1.24, 95% confidence interval [CI] = 0.68 to 2.26). A family history of breast cancer in a female relative was not associated with an increased risk of colorectal cancer, even after adjustment for the presence of a BRCA founder mutation (OR = 1.03, 95% CI = 0.75 to 1.41). Conclusions: Although weak associations cannot be excluded, Ashkenazi BRCA founder mutations do not confer a strongly elevated risk of colorectal cancer. Similarly, a family history of breast cancer does not appear to be a strong risk factor for colorectal cancer in this population.

Background: Bladder cancer is characterized by genomic instability. In this study, we investigated whether genome-wide screening using single-nucleotide polymorphism (SNP) arrays could detect allelic imbalance (loss or gain of at least one allele) in bladder cancers. Methods: For microarray analysis, DNA was isolated from microdissected bladder tumors and leukocytes from 11 patients. The stage T1 tumor (connective tissue invasive) and the subsequent stage T2-4 tumor (muscle invasive) were available from eight of these patients, and only the first muscle-invasive stage T2-4 tumor was available from three of the 11 patients. The microarray contained 1494 biallelic polymorphic sequences. For microsatellite analyses, DNA was isolated from tumors and leukocytes of nine patients with primary T2-4 tumors and 13 patients with Ta (noninvasive) tumors. All statistical tests were two-sided. Results: We assigned a genotype to 1204 loci, 343 of which were heterozygous. Allelic imbalance was detected in known areas of imbalance on chromosomes 6, 8, 9, 11, and 17, and a new area of imbalance was detected on the p arm of chromosome 6. Microsatellite analysis of nine other T2-4 tumors and 13 Ta tumors showed that allelic imbalance was more frequent in T2-4 tumors than in Ta tumors (P<.001). We detected 8.5 allelic imbalances (median) in 348 informative loci in T1 tumors and 28 allelic imbalances (median) in 329 informative loci in T2-4 tumors. When pairs of T1 and T2-4 tumors were analyzed from eight patients, 68% of imbalances detected in T1 tumors (146 imbalances) occurred in the subsequent T2-4 tumors (99 imbalances). Homozygous TP53 mutations were more often associated (P = .005) with high allelic imbalance than with low allelic imbalance. Conclusion: SNP arrays are feasible for high-throughput, genome-wide scanning for allelic imbalances in bladder cancer.

Background: Cervical carcinogenesis is initiated by infection with high-risk (i.e., carcinogenic)
human papillomavirus (HPV) types. The subsequent progression from premalignant cervical intraepithelial neoplasia (CIN) to invasive cancer is driven by both genetic and epigenetic processes. We assessed the role of the gene encoding the adhesion molecule tumor suppressor in lung cancer 1 (TSLC1) in this progression. Methods: We analyzed TSLC1 gene expression by real-time quantitative reverse transcription-polymerase chain reaction, promoter methylation by sodium bisulfite genomic DNA sequencing, and allelic loss by microsatellite analysis in primary keratinocytes, in four non-tumorigenic HPV-immortalized human keratinocyte cell lines, and in 11 human cervical cancer cell lines that were positive for a high-risk HPV DNA type and in normal cervical epithelial cells. We transfected cervical cancer SiHa cells that did not express TSLC1 mRNA with an expression vector containing the TSLC1 complementary DNA (cDNA) or an empty vector and analyzed transfectants for anchorage-independent growth and tumorigenicity in nude mice. We also examined TSLC1 promoter methylation in premalignant cervical lesions and in cervical carcinomas and smears. All statistical tests were two-sided. Results: TSLC1 mRNA was strongly reduced, relative to levels in primary keratinocytes, or absent in 10 (91%) of 11 cervical carcinoma cell lines but in none (0%) of the four HPV-immortalized cell lines (difference = 91%, 95% confidence interval [CI] = 74% to 100%; P =.004). The TSLC1 promoter was hypermethylated, relative to normal foreskin and cervical epithelial cells, in nine (82%) of the 11 cervical carcinoma cell lines but in none (0%) of the four HPV-immortalized cell lines (difference = 82%, 95% CI = 59% to 100%; P =.01). Seven (88%, 95% CI = 47% to 100%) of the eight SiHa/TSLC1 transfectants displayed a marked reduction in anchorage-independent growth (i.e., 0-100 colonies per 5000 cells) compared with none of the four (0%, 95% CI = 0% to 60%) SiHa transfectants bearing the empty vector (i.e., SiHa/hygro transfectants; difference = 88%, 95% CI = 65% to 100%; P =.01) or untransfected SiHa cells. All seven mice (100%, 95% CI = 59% to 100%) injected with untransfected SiHa cells or SiHa/hygro transfectants displayed tumors of at least 50 mm3 by 2-6 weeks after injection compared with none of eight mice (0%, 95% CI = 0% to 37%) injected with untransfected SiHa cells or SiHa/hygro transfectants displayed tumors of at least 50 mm3 by 2-6 weeks after injection compared with none of eight mice (0%, 95% CI = 0% to 37%) injected with the SiHa/TSLC1 transfectants (difference = 100%, 95% CI = 68% to 100%; P<.001). We detected TSLC1 promoter hypermethylation in seven (35%, 95% CI = 15% to 59%) of 20 high-grade CIN lesions (i.e., CIN II and III) and in 30 (58%, 95% CI = 43% to 71%) of 52 cervical squamous cell carcinomas compared with none (0%, 95% CI = 0% to 34%) of nine normal cervical epithelial biopsy samples and none (0%, 95% CI = 0% to 22%) of 12 CIN I lesions (P<.001 for cervical squamous cell cancer versus normal epithelial biopsy samples plus CIN I lesions). Conclusions: TSLC1 gene silencing via promoter hypermethylation is a frequent event in the progression from high-risk HPV-containing, high-grade CIN lesions to invasive cervical cancer.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;97/8/577

Background: Little is known in human immunodeficiency virus (HIV)-positive women about how the combination of plasma HIV RNA level and CD4+ T-cell count is associated with the natural history of human papillomavirus (HPV) infection or about HPV reactivation—whether it occurs and with what frequency in HIV-positive women. Methods: HIV-positive (n = 1848) and -negative (n = 514) women were assessed at semiannual visits (total person-years = 5661) for cervicovaginal HPV with polymerase chain reaction assays and for squamous intraepithelial lesions (SILs) by Pap smear. We studied the prevalent detection of HPV and SILs with generalized estimating equations and the incident detection and persistence of HPV and SILs with multivariable Cox models. All statistical tests were two-sided. Results: We observed a strong interaction between the associations of CD4+ and plasma HIV RNA strata with both prevalent (Pinteraction =.002) and incident (Pinteraction =.001) detection of HPV. Indeed, the hazard ratio for incident HPV detection peaked between 4.0 and 5.0, with either a CD4+ count of less than 200 cells per mm3 or an HIV RNA level of more than 100 000 copies per mL. Although incident HPV detection in all
women was associated with the number of recent sex partners (Ptrend<.001), 22% of sexually inactive HIV-positive women with a CD4+ count of less than 200 cells/mm3 also had at least one incidently detected HPV type. The association between CD4+/HIV RNA strata and HPV persistence was statistically significantly smaller (P<.001) than for incident HPV detection. SIL prevalence, incident detection, and persistence had similar associations with CD4+/HIV RNA strata as HPV (above). Conclusion: In HIV-positive women, plasma HIV RNA level and CD4+ count in combination appear to have a strong and statistically interactive association with incident detection of HPV, some of which may reflect HPV reactivation (e.g., in sexually inactive women). The more moderate association between HIV coinfection and HPV persistence could partly explain why cervical cancer rates have not reached more epidemic proportions in HIV-positive women.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;94/8/617

Background: Reproductive hormones are associated with risk for epithelial ovarian cancer. To determine the effect of such hormones on the activation of interleukin 6 (IL-6)/STAT3 (signal transducer and activator of transcription-3) signaling, which may be involved in ovarian cancer, we investigated the status of STAT3, IL-6, and its receptor in immortalized human ovarian surface epithelial (HOSE) and ovarian cancer (OVCA) cell lines. Methods: Two immortalized HOSE cell lines and two OVCA cell lines were cultured with gonadotropins, sex steroid hormones, and/or IL-6, alone or with specific inhibitors or IL-6-neutralizing antibodies. Expression of IL-6, the IL-6 receptor (alpha) chain (IL-6R(α)), and phosphorylated and unphosphorylated STAT3 messenger RNAs (mRNAs) and proteins in all cells was determined. Cell proliferation and soft-agar colony formation were assessed. STAT3 activity was investigated in OVCA cells transfected with a dominant negative STAT3 (Dn-STAT3), wild-type STAT3, or an empty control vector. All statistical tests were two-sided. Results: Levels of IL-6 mRNA and protein increased in all cells treated with follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol, or estrone but increased only in OVCA cells treated with testosterone and 5[alpha]-dihydrotestosterone. For all lines, IL-6 antibodies partially inhibited hormone-stimulated cell proliferation but completely inhibited IL-6-enhanced cell proliferation. IL-6 induced STAT3 phosphorylation and activation in HOSE cells; STAT3 was constitutively activated in OVCA cells. Higher levels of IL-6R(α) and STAT3 transcription factors were observed in OVCA cells than in HOSE cells. After transfection, Dn-STAT3 suppressed endogenous STAT3 and inhibited all forms of IL-6-stimulated OVCA cell proliferation (OVCA 429 cells, P<.001; OVCA 432 cells, P<.006), whereas wild-type STAT3 enhanced HOSE cell proliferation (wild-type STAT3 at 0.5 {micro}g/mL in HOSE 306 cells, P<.002; STAT3 at 1.0 {micro}g/mL in HOSE 306 or both concentrations of wild-type STAT3 in HOSE 642 cells, P<.001). Conclusions: The IL-6/STAT3 signaling pathway may mediate FSH-, LH-, and estrogen-stimulated HOSE cell proliferation. Increased IL-6R(α) expression and constitutive STAT3 activation may be associated with ovarian cancer.


http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/9/669

Background: Rothmund-Thomson syndrome (RTS) is an autosomal recessive disorder
associated with an increased predisposition to osteosarcoma. Children with RTS typically present with a characteristic skin rash (poikiloderma), small stature, and skeletal dysplasias. Mutations in the RECQL4 gene, which encodes a RecQ DNA helicase, have been reported in a few RTS patients. We examined whether a predisposition to developing osteosarcoma among an international cohort of RTS patients was associated with a distinctive pattern of mutations in the RECQL4 gene. Methods: We obtained clinical information about and biologic samples from 33 RTS patients (age range = 1-30 years). Eleven patients were diagnosed with osteosarcoma. All 21 exons and 13 short introns of the RECQL4 gene were sequenced from the genomic DNA of all subjects. Kaplan-Meier survival analysis was used to estimate the incidence of osteosarcoma among patients with and without mutations predicted to produce a truncated RECQL4 protein. Results: Twenty-three RTS patients, including all 11 osteosarcoma patients, carried at least one of 19 truncating mutations in their RECQL4 genes. The incidence of osteosarcoma was 0.00 per year in truncating mutation-negative patients (100 person-years of observation) and 0.05 per year in truncating mutation-positive patients (230 person-years of observation) (P =.037; two-sided log-rank test). Conclusions: Mutations predicted to result in the loss of RECQL4 protein function occurred in approximately two-thirds of RTS patients and are associated with risk of osteosarcoma. Molecular diagnosis has the potential to identify those children with RTS who are at high risk of this cancer.

J Neurophysiol (2)


http://jn.physiology.org/cgi/content/abstract/90/1/155

When studied in vitro, type I hair cells in amniote vestibular organs have a large, negatively activating K+ conductance. In type II hair cells, as in nonvestibular hair cells, outwardly rectifying K+ conductances are smaller and more positively activating. As a result, type I cells have more negative resting potentials and smaller input resistances than do type II cells; large inward currents fail to depolarize type I cells above -60 mV. In nonvestibular hair cells, afferent transmission is mediated by voltage-gated Ca2+ channels that activate positive to -60 mV. We investigated whether Ca2+ channels in type I cells activate more negatively so that quanetal transmission can occur near the reported resting potentials. We used the perforated patch method to record Ca2+ channel currents from type I and type II hair cells isolated from the rat anterior crista (postnatal days 4-20). The activation range of the Ca2+ currents of type I hair cells differed only slightly from that of type II cells or nonvestibular hair cells. In 5 mM external Ca2+, currents in type I and type II cells were half-maximal at -41.1 +/- 0.5 (SE) mV (n = 10) and -37.2 +/- 0.2 mV (n = 10), respectively. In physiological external Ca2+ (1.3 mM), currents in type I cells were half-maximal at -46 +/- 1 mV (n = 8) and just 1% of maximal at -72 mV. These results lend credence to suggestions that type I cells have more positive resting potentials in vivo, possibly through K+ accumulation in the synaptic cleft or inhibition of the large K+ conductance. Ca2+ channel kinetics were also unremarkable; in both type I and type II cells, the currents activated and deactivated rapidly and inactivated only slowly and modestly even at large depolarizations. The Ca2+ current included an L-type component with relatively low sensitivity to dihydropyridine antagonists, consistent with the {alpha} subunit being CaV1.3 ({alpha}1D). Rat vestibular epithelia and ganglia were probed for L-type {alpha}-subunit expression with the reverse transcription-polymerase chain reaction. The epithelia expressed CaV1.3 and the ganglia expressed CaV1.2 ({alpha}1C).

http://jn.physiology.org/cgi/content/abstract/89/5/2499

Distler, C., P. K. Rathee, K. S. Lips, O. Obreja, W. Neuhuber, and M. Kress. Fast Ca2+-Induced Potentiation of Heat-Activated Ionic Currents Requires cAMP/PKA Signaling and Functional AKAP Anchoring. J. Neurophysiol. 89: 2499-2505, 2003. Calcium influx and the resulting increase in intracellular calcium concentration ([Ca2+]i) can induce enhanced sensitivity to temperature increases in nociceptive neurons. This sensitization accounts for heat hyperalgesia that is regularly observed following the activation of excitatory inward currents by pain-producing mediators. Here we show that rat sensory neurons express calcium-dependent adenylyl cyclases (AC) using RT-PCR and nonradioactive in situ hybridization. Ionomycin-induced rises in [Ca2+]i-activated calcium-dependent AC and caused translocation of catalytic protein kinase A subunit. Elevation of [Ca2+]i finally resulted in a significant potentiation of heat-activated currents and a drop in heat threshold. This was not prevented in the presence of suramin that nonspecifically uncouples G protein-dependent receptors. The sensitization was, however, inhibited when the specific PKA antagonist PKI14-22 was added to the pipette solution or when PKA coupling to A kinase anchoring protein (AKAP) was disrupted with InCELLect StHt-31 uncoupling peptide. The results show that heat sensitization in nociceptive neurons can be induced by increases in [Ca2+]i and requires PKA that is functionally coupled to the heat transducer, mostly likely vanilloid receptor VR-1. This calcium-dependent pathway can account for the sensitizing properties of many excitatory mediators that activate cationic membrane currents.

J Soc Biol (1)


In this study, we have evaluated the efficacy and the validity of the AmpFISTR SGM plus multiplex PCR typing system when Low Copy Number (LCN) amounts of DNA are processed. The characteristics of SGM plus profiles produced under LCN conditions were studied on the basis of heterozygote balance, between loci balance and stutter proportion based on profiles that were obtained from a variety of mock casework samples. These experiments clearly showed that LCN DNA profiles carry their own characteristic features, which must be taken into account during interpretation. Herewith, we confirmed the data of recent other studies that a comprehensive interpretation strategy is dependent upon multiple replication of the PCR using the same extract together with the proper use of extraction and amplification controls. The limitations of LCN DNA analysis were further studied in a series of single cell PCR experiments using an amplification regime of 34 PCR cycles. The allele dropout phenomenon was demonstrated to its full extent when single cells were analysed. However, the "consensus profile" which was obtained from separate single cell PCR experiments matched the actual profile of the cell donor. Single cell PCR experiments also showed that a further increase of the number of PCR cycles did not result in enhanced sensitivity and had a highly negative effect on the balance of this multiplex PCR.
system which hampered correct interpretation of the profile. Also, the potential of LCN typing in analysing mixtures of DNA was investigated. It was clearly shown that LCN typing had no advantages over 28 cycles amplification in the detection of the minor component of DNA-mixtures. In addition to the 34 cycles PCR amplification regime, the utility of a new approach that involved reamplification of the 28 cycle SGM plus PCR products with an extra 6 PCR cycles after the addition of fresh AmpliTaq Gold DNA Polymerase was investigated. This approach provides the scientist with an extra typing result that enhances the reliability of the consensus profile, which is commonly retrieved from two separate 34 cycle PCR results. Furthermore, the 28 + 6 cycles approach may be used to screen LCN samples for their potential to produce a 34 PCR cycle profile. Finally and as a last resort the 28 + 6 cycles approach can be used in those cases where no further extract from the crime sample is available. Finally, the potential of LCN typing was demonstrated in typing samples from non-probative and actual casework examples. From a high proportion of samples that failed to demonstrate SGM plus typing results using the standard protocol of 28 cycles, at least partial profiles could be obtained after LCN methods were used. For example, LCN typing was applied in a case where 10-year old samples from bones and teeth that were retrieved from a mass grave had to be identified. This study resulted in the positive identification of a number of victims by comparing the LCN DNA profiles with the profiles from putative relatives. The value of LCN DNA typing was further demonstrated in a strangulation case. The throat of the victim was sampled and only after 34 PCR cycles were we able to reveal that the evidential sample contained a distinct mixture of the victim's own DNA and the DNA of the defendant.

J. Am. Soc. Nephrol. (26)


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ABSTRACT. Slowly progressive renal injury is the major cause for ESRD. The model of progressive immune complex glomerulonephritis in autoimmune MRL/lpr/lpr mice was used to evaluate whether chemokine receptor CCR1 blockade late in the disease course can affect progression to renal failure. Mice were treated with subcutaneous injections of either vehicle or BX471, a nonpeptide CCR1 antagonist, three times a week from week 20 to 24 of age. BX471 improved blood urea nitrogen levels (BX471, 35.1 {+/-} 5.3; vehicle, 73.1 {+/-} 39.6 mg/dl; P < 0.05) and reduced the amount of ERH-3 macrophages, CD3 lymphocytes, Ki-67 positive proliferating cells, and ssDNA positive apoptotic cells in the interstitium but not in glomeruli. Cell transfer studies with fluorescence-labeled T cells that were pretreated with either vehicle or BX471 showed that BX471 blocks macrophage and T cell recruitment to the renal interstitium of MRL/lpr/lpr mice. This was associated with reduced renal expression of CC chemokines CCL2, CCL3, CCL4, and CCL5 and the chemokine receptors CCR1, CCR2, and CCR5. Furthermore, BX471 reduced the extent of interstitial fibrosis as evaluated by interstitial smooth muscle actin expression and collagen I deposits, as well as mRNA expression for collagen I and TGF-(beta). BX471 did not affect serum DNA autoantibodies, proteinuria, or markers of glomerular injury in MRL/lpr/lpr mice. This is the first evidence that, in advanced chronic renal injury, blockade of CCR1 can halt disease progression and improve renal function by selective inhibition of interstitial leukocyte recruitment and fibrosis.

http://www.jasn.org/cgi/content/abstract/14/7/1889

ABSTRACT. The immunosuppressive drug tacrolimus, whose pharmacokinetic characteristics display large interindividual variations, is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance-1 (MDR1) gene. Some of the single nucleotide polymorphisms (SNP) of MDR1 reported correlated with the in vivo activity of P-gp. Because P-gp is known to control tacrolimus intestinal absorption, it was postulated that these polymorphisms are associated with tacrolimus pharmacokinetic variations in renal transplant recipients. The objective of this study was to evaluate in a retrospective study of 81 renal transplant recipients the effect on tacrolimus dosages and concentration/dose ratio of four frequent MDR1 SNP possibly associated with P-gp function (T-129C in exon 1b, 1236C>T in exon 12, 2677G>T,A in exon 21, and 3435C>T in exon 26). As in the general population, the SNP in exons 12, 21, and 26 were frequent (16, 17.3, and 22.2% for the variant homozygous genotype, respectively) and exhibited incomplete linkage disequilibrium. One month after tacrolimus introduction, exon 21 SNP correlated significantly with the daily tacrolimus dose (P [<=] 0.05) and the concentration/dose ratio (P [<=] 0.02). Tacrolimus dose requirements were 40% higher in homozygous than wild-type patients for this SNP. The concentration/dose ratio was 36% lower in the wild-type patients, suggesting that, for a given dose, their tacrolimus blood concentration is lower. Haplotype analysis substantiated these results and suggested that exons 26 and 21 SNP may be associated with tacrolimus dose requirements. Genotype monitoring of the MDR1 gene reliably predicts the optimal dose of tacrolimus in renal transplant recipients and may predict the initial daily dose needed by individual patients to obtain adequate immunosuppression. E-mail: Dany.Anglicheau@biomedicale.univ-paris5.fr


http://www.jasn.org/cgi/content/abstract/15/10/2666

In experimental and human renal diseases, progression is limited by angiotensin-converting enzyme inhibitors. Whether renoprotection was due to their capacity of reducing proinflammatory and profibrotic effects of angiotensin II (Ang II) or limiting proteinuria and its long term toxicity is debated. For dissecting the relative contribution of Ang II and proteinuria to chronic renal damage, the protein-overload proteinuria model was used in genetically modified mice lacking the major isoform of murine AT1 receptor (AT1A). Uninephrectomized AT1A+/+ and -/- mice received a daily injection of BSA or saline for 4 or 11 wk. AT1A-/-BSA mice acquired a renal phenotype of proteinuria and renal glomerular and tubulointerstitial lesions, albeit attenuated with respect to AT1A+/+BSA. Administration of the calcium channel blocker lacidipine to reduce BP of AT1A+/+BSA mice to levels of AT1A-/-BSA translated into comparable values of protein excretion rate and glomerular and tubulointerstitial injury in both strains. These results confirm that the toxic effect of protein trafficking on renal disease progression is not necessarily dependent on Ang II to the extent that targeted deletion of AT1A does not prevent disease progression. A role of Ang II via AT1B or AT2 receptors is still a possibility that cannot be ruled out by the present experimental approach. These findings provide a clear rationale for specifically targeting proteinuria in pharmacologic interventions of chronic nephropathies.

ABSTRACT. Leukocyte infiltration of the cortico-interstitium is characteristic of many forms of progressive renal disease. The principal adhesion molecule expressed on resident interstitial cells and recognized by leukocytes is intercellular adhesion molecule-1 (ICAM-1). ICAM-1 is an inducible transmembrane receptor, which forms the counter-receptor for the leukocyte (beta)2 integrins. ICAM-1-dependent binding induces the synthesis of the chemokine RANTES and of ICAM-1 itself. This study examines some of the signaling pathways involved in this induction. After ICAM-1 cross-linking on fibroblasts, the mRNA and protein for both RANTES and ICAM-1 were induced. This induction was calcium-dependent and inhibited by BAPTA-AM. The p38, ERK1, and ERK2 MAP kinases were activated in a [Ca2+]i-dependent manner, with a maximum phosphorylation at approximately 3 min after cross-linking. Through the use of selective inhibitors of p38 MAP kinase (SB203580) or MEKK (PD98059), p38 but not ERK activation was shown to be essential for the induction of ICAM-1. Neither was involved in RANTES activation, however. These mechanisms differed from those initiated by TNF-(alpha), which were not [Ca2+]i-dependent. Electrophoretic mobility shift analysis demonstrated a time-dependent induction of both AP-1 and NF-(kappa)B binding activity in nuclear extracts, maximal at approximately 15 min after ICAM-1 cross-linking. Only AP-1 activation, however, was calcium-dependent, suggesting the central involvement of this transcription factor in ICAM-1 and RANTES induction after the ligation of ICAM-1. This study suggests an independent mechanism of inflammatory amplification, which may be characteristic of a persistent leukocytic involvement in areas of chronic inflammation rather than in cytokine-induced acute inflammation. E-mail: Steadmanr@cf.ac.uk


ABSTRACT. Familial juvenile hyperuricemic nephropathy (FJHN [MIM 162000]) is an autosomal-dominant disorder characterized by abnormal tubular handling of urate and late development of chronic interstitial nephritis leading to progressive renal failure. A locus for FJHN was previously identified on chromosome 16p12 close to the MCKD2 locus, which is responsible for a variety of autosomal-dominant medullary cystic kidney disease (MCKD2). UMOD, the gene encoding the Tamm-Horsfall/uromodulin protein, maps within the FJHN/MCKD2 critical region. Mutations in UMOD were recently reported in nine families with FJHN/MCKD2 disease. A mutation in UMOD has been identified in 11 FJHN families (10 missense and one in-frame deletion)—10 of which are novel—clustering in the highly conserved exon 4. The consequences of UMOD mutations on uromodulin expression were investigated in urine samples and renal biopsies from nine patients in four families. There was a markedly increased expression of uromodulin in a cluster of tubule profiles, suggesting an accumulation of the protein in tubular cells. Consistent with this observation, urinary excretion of wild-type uromodulin was significantly decreased. The latter findings were not observed in patients with FJHN without UMOD mutations. In conclusion, this study points to a mutation clustering in exon 4 of UMOD as a major genetic defect in FJHN. Mutations in UMOD may critically affect the function of uromodulin, resulting in abnormal accumulation within tubular cells and reduced urinary excretion. E-mail: Dahan@gmed.ucl.ac.be

ABSTRACT. PDGF-C is a new member of the PDGF-family and has recently been identified as a rat mesangial cell mitogen. Its expression and function in human kidneys is unknown. Localization of PDGF-C protein was analyzed by immunohistochemistry using a rabbit polyclonal antibody directed against the core-domain of PDGF-C in human fetal kidneys (n = 8), normal adult human kidneys (n = 9), and in renal biopsies of patients with IgA nephropathy (IgAN, n = 31), membranous nephropathy (MGN, n = 8), minimal change disease (MC, n = 7), and transplant glomerulopathy (TxG, n = 12). Additionally, PDGF-C mRNA was detected in microdissected glomeruli by real-time RT-PCR in cases of normal adult kidneys (n = 7), IgAN (n = 27), MGN (n = 11), and MC (n = 13). In the fetal kidney, PDGF-C localized to the developing mesangium, ureteric bud epithelium, and the undifferentiated mesenchyme. In the adult kidney, PDGF-C was constitutively expressed in parietal epithelial cells of Bowman's capsule, tubular epithelial cells (loops of Henle, distal tubules, collecting ducts), and in arterial endothelial cells. A marked upregulation of glomerular PDGF-C protein was seen in MGN and TxG with a prominent positivity of virtually all podocytes. In MC, PDGF-C localized to podocytes in a more focal distribution. In MGN, increased glomerular PDGF-C protein expression was due to increased mRNA synthesis as a 4.3-fold increase in PDGF-C mRNA was detected in microdissected glomeruli from MGN compared with normal. PDGF-C protein was additionally expressed in individual mesangial cells in TxG. Finally, upregulated PDGF-C protein expression was detected within sclerosing glomerular and fibrosing tubulointerstitial lesions in individual cases from all analyzed groups. We conclude that PDGF-C is constitutively expressed in the human kidney and is upregulated in podocytes and interstitial cells after injury/activation of these cells. E-mail: feitner@ukaachen.de

ABSTRACT. Autosomal recessive polycystic kidney disease (ARPKD; MIM 263200) is a hereditary and severe form of polycystic disease affecting the kidneys and biliary tract with an estimated incidence of 1 in 20,000 live births. The clinical spectrum is widely variable: up to 50% of affected neonates die shortly after birth, whereas others survive to adulthood. Mutations at a single locus, polycystic kidney and hepatic disease 1 (PKHD1), are responsible for all typical forms of ARPKD. Mutation detection was performed in PKHD1 by DHPLC in 85 affected, unrelated individuals. Seventy-four amplicons were amplified and analyzed from the PKHD1 genomic locus. Sequence variants were considered pathogenic when they were not observed in 160 control individuals (320 chromosomes). For purposes of genotype-phenotype comparisons, families were stratified by clinical presentation into two groups: the severe perinatal group, in which at least one affected child presented with perinatal disease and neonatal demise, and the less severe, nonperinatal group, in which none of the affected children died in the neonatal period. Forty-one mutations were found in 55 affected disease chromosomes; 32 of these mutations have not been reported previously. Mutations were distributed throughout the portions of gene encoding the predicted extracellular portion of the protein product. The most commonly encountered mutation, T36M, was found in 8 of 55 disease chromosomes. Amino acid substitutions were found to be more commonly associated with a nonlethal presentation, whereas chain terminating mutations were more commonly associated with neonatal demise ($\chi^2 = 11.54, P = 0.003$). All patients who survive the neonatal period have at least one amino acid substitution mutation, suggesting that such substitutions produce milder disease through production of partially functional protein products. The nature of the germline mutations in ARPKD plays a significant role in determining clinical outcome. E-mail: lgw@uab.edu


ABSTRACT. Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disorder characterized by focal cyst formation from any part of the nephron. The molecular bases include germinal mutation of either PKD1 or PKD2 genes, enhanced expression of several protooncogenes, alteration of the TGF-(alpha)/EGF/EGF receptor (EGFR) axis, and disturbed regulation of proliferative/apoptosis pathways. To identify new locations of ADPKD related oncogenes and/or tumor suppressor genes (TSG), comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analyses were performed for a series of individual cysts (n = 24) from eight polycystic kidneys. By CGH, imbalances were detected predominantly on chromosomes 1p, 9q, 16p, 19, and 22q in all tissues. DNA copy number gain was seen on chromosomes 3q and 4q in five samples. The CGH data were supplemented by LOH analysis using 83 polymorphic microsatellite markers distributed along chromosomes 1, 9, 16, 19, and 22. The highest frequency of LOH was found on the 1p35-36 and 16p13.3 segments in cysts from seven samples. Allelic losses on 9q were detected in six, whereas deletions at 19p13 and 22q11 bands were observed in three polycystic kidneys. These results indicate that the deleted chromosomal regions may contain genes important in ADPKD initiation and progression. E-mail: gogusev@necker.fr

Grandaliano, G., P. Pontrelli, et al. (2003). "Protease-Activated Receptor-2 Expression in IgA
ABSTRACT. An increasing body of evidence suggests that proteases may play a key role in the pathogenesis of tissue fibrosis. Protease-activated receptor-2 (PAR-2) is cleaved and activated by trypsin-like proteolytic enzymes, including tryptase and activated coagulation factor X (FXa). Both these soluble mediators have been demonstrated, directly or indirectly, at the interstitial level in progressive renal diseases, including IgA nephropathy (IgAN). PAR-2 mRNA and protein levels were investigated by RT-PCR and immunohistochemistry, respectively, in 17 biopsies from IgAN patients and 10 normal kidneys. PAR-2 expression was also evaluated, by RT-PCR and western blotting, in cultured human mesangial and proximal tubular cells. Finally, gene expression of plasminogen activator inhibitor-1 (PAI-1) and TGF-β, two powerful fibrogenic factors, was evaluated in FXa-, trypsin-, and PAR-2 activating peptide-stimulated human proximal tubular cells by Northern blot. In normal kidneys, PAR-2 gene expression was barely detectable, whereas in IgAN biopsies the mRNA levels for this protease receptor were strikingly increased and directly correlated with the extent of interstitial fibrosis. Immunohistochemical staining demonstrated that PAR-2 protein expression in IgAN biopsies was mainly localized in the proximal tubuli and within the interstitial infiltrate. Proximal tubular cells in culture expressed PAR-2. Activation of this receptor by FXa in tubular cells induced a striking increase in intracellular calcium concentration. In addition, incubation of both cell lines with trypsin, FXa, or PAR-2 activating peptide caused a marked upregulation of PAI-1 gene expression that was not counterbalanced by an increased expression of plasminogen activators. Finally, PAR-2 activation induced a significant upregulation of TGF-β gene and protein expression in both mesangial and tubular cells. On the basis of our data, we can suggest that PAR-2 expressed by renal resident cells and activated by either mast cell tryptase or FXa may induce extravascular matrix deposition modifying the PAI-1/PA balance and inducing TGF-β expression. These molecular mechanisms may underlie interstitial fibrosis in IgAN. E-mail: g.grandaliano@nephro.uniba.it


ABSTRACT. The directed migration of cells, cell-cell adhesion, and the control of proliferation are key events during metanephric development. The chemokines are a family of proteins that selectively control aspects of cell migration, activation, proliferation, and adhesion. The expression of a series of chemokines and chemokine receptors during human renal development was investigated by using immunohistochemical analyses and real-time reverse transcription-PCR assays of defined laser-microdissected metanephric structures. The results demonstrate that mononuclear cell-like cells within the nephrogenic blastema focally express interferon-inducible protein-10/CXCL10, a ligand for CXCR3. Mononuclear-like cells dispersed through the developing organ express CX3CR1. Expression of CXCR4, the receptor for stromal cell-derived factor-1/CXCL12, is also limited to stromal CD34-positive cells. In contrast, the expression of stromal cell-derived factor-1/CXCL12, fractalkine, and CXCR3 is first observed in the comma- or S-shaped body stage. The intensity of this expression becomes stronger in the capillary loop stage, and expression is mainly observed in the mesangial stalk and endothelial cells of the glomeruli. These proteins may play modulatory roles in kidney development. Because genes that are expressed during ontogeny often play a role in tissue regeneration, these embryonal chemokine/chemokine receptor patterns may be important in renal injury and repair.

http://www.jasn.org/cgi/content/abstract/14/8/2099

ABSTRACT. The A3243G mutation of the mitochondrial tRNA(Leu) gene has been recently reported in rare patients with focal and segmental glomerulosclerosis (FSGS). However, the full spectrum of systemic and kidney manifestations in adults presenting with this mutation remains poorly defined. Assessment of renal and nonrenal manifestations was performed in nine patients with A3243G mutation and prominent kidney disease diagnosed in adulthood. At first renal evaluation, median age was 35 years. Renal lesions consisted of FSGS (n = 2), tubulointerstitial nephropathy (n = 3), or bilateral enlarged cystic kidneys (n = 1). All but one patient exhibited extrarenal manifestations: deafness (8 of 9) requiring hearing aid in half the cases, diabetes mellitus (3 of 9), neuromuscular involvement (2 of 9), hypertrophic cardiomyopathy (1 of 9), and macular dystrophy (1 of 9). After a median follow-up of 5 yr, five patients progressed to end-stage renal disease between the ages of 15 and 51 years, four being successfully transplanted. Similarly, extrarenal manifestations progressed since all patients had deafness and diabetes (including three posttransplants), while half had neuromuscular, cardiac, or retinal involvement. In the adult patients with A3243G mutation and renal involvement, preexisting deafness is almost consistently found. While FSGS remains the most typical lesion, tubulointerstitial nephropathy or bilateral, enlarged cystic kidneys may also be encountered. In most cases, diabetes mellitus, macular dystrophy, hypertrophic cardiomyopathy, or neuromuscular features occur later in the course of the disease. The severity of the clinical course is heterogeneous, with end-stage renal failure being reached between the second and sixth decades. Renal transplantation may be offered to these patients, despite a high incidence of steroid-induced diabetes mellitus. E-mail: dominique.chauveau@nck.ap-hop-paris.fr


http://www.jasn.org/cgi/content/abstract/15/8/2229

ABSTRACT. Genotype DD of the angiotensin-converting enzyme (ACE) is not associated with an increased incidence of native renal diseases, although it could modulate progression to renal failure in patients who already display chronic lesions. Because its role in renal allograft degeneration is not well characterized, whether ACE genotype was associated with the prevalence of chronic allograft nephropathy (CAN) was studied, in a group of protocol biopsies from 180 patients, or with the incidence of CAN in 152 patients with at least two sequential biopsies. As a control group, ACE genotype was also studied in 41 donors and 72 healthy subjects. For analyzing the influence of ACE genotype in graft survival, patients were grouped into six categories (II-normal biopsy, ID-normal, DD-normal, II-CAN, ID-CAN and DD-CAN). Finally, relative renal ACE mRNA levels were measured in 67 cases by real-time PCR using the delta threshold cycle method. ACE-DD genotype was more frequent in patients who received a transplant than in control subjects (43.3% versus 30.1%, P = 0.026), but prevalence (DD = 42.7% versus non-DD = 42.2%) or incidence (DD = 24.6% versus non-DD = 29.9%) of CAN was not different regarding recipient ACE genotype. Furthermore, patients with the ACE-DD genotype and CAN had the poorest graft survival (II-normal = 100%, ID-normal = 91%, DD-normal = 84%, II-CAN = 100%, ID-CAN = 66%, and DD-CAN = 36%; P = 0.034) and higher ACE mRNA levels than non-DD and CAN (DD = -3.36 +/- 2.35 versus non-DD = -5.65 +/- 1.72-fold in ACE copies; P = 0.012). It is concluded that ACE-DD genotype is not associated with an increased prevalence or incidence of CAN but is actually associated with higher ACE mRNA levels and poorer graft survival in patients who already display CAN.
ABSTRACT. Indirect X chromosome-inactivation analyses have demonstrated that most parathyroid glands from patients with uremic refractory secondary/tertiary hyperparathyroidism are monoclonal neoplasms. However, little is known regarding the specific acquired genetic abnormalities that must underlie such clonal expansion or the molecular pathogenetic features of this disorder, compared with primary parathyroid adenomas. To address these issues in a uniquely powerful manner, both comparative genomic hybridization (CGH) and genome-wide molecular allelotyping were performed with a large group of uremia-associated parathyroid tumors. As indicated by CGH, one or more chromosomal changes were present in 24% of the tumors, which is markedly different from the value for common sporadic adenomas (72%). Two recurrent abnormalities that had not been previously described for sporadic parathyroid adenomas were noted with CGH, i.e., gains on chromosomes 7 (9%) and 12 (11%). Losses on chromosome 11 occurred in only one of the 46 uremia-associated tumors (2%); the tumor also contained a somatic mutation of the remaining MEN1 allele (221del18). A total of 13% of tumors demonstrated recurrent allelic loss on 18q, with 18q21.1-q21.2 being defined as the putative tumor suppressor-containing region. In conclusion, the powerful combination of genome-wide molecular allelotyping and CGH has identified recurrent clonal DNA abnormalities that suggest the existence and locations of genes important in uremic hyperparathyroidism. In addition, genome-wide patterns of somatic DNA alterations, including disparate roles for MEN1 gene inactivation, indicate that markedly different molecular pathogenetic processes exist for clonal outgrowth in severe uremic hyperparathyroidism versus common parathyroid adenomas.

ABSTRACT. The extent of graft damage after ischemia-reperfusion reflects the balance between deleterious events and protective factors. Heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF) may contribute to cytoprotection by their anti-inflammatory and antiapoptotic properties. For investigating whether HO-1 and VEGF play a role in the adaptive response to ischemia-reperfusion injury after renal transplantation, kidney biopsies were analyzed from living (n = 45) and cadaveric (n = 16) donors, obtained at three time points: at the end of cold storage T(-1), after warm ischemia T(0), and after reperfusion T(+1). The mRNA expression levels of HO-1, VEGF165, Bcl-2, Bax, and hypoxia inducible factor-1{alpha} were quantified by real-time reverse transcriptase-PCR, and the HO-1 and VEGF proteins were analyzed by immunohistochemistry.Cadaveric donor kidneys presented higher mRNA expression levels of hypoxia inducible factor-1{alpha}. In contrast, mRNA expression levels of HO-1, VEGF165, and Bcl-2 were significantly lower in kidneys from cadaveric donors. Overall, a significant correlation was observed between mRNA expression of Bcl-2 and VEGF165, between Bcl-2 and HO-1, and between HO-1 and VEGF165. Moreover, protein expression of HO-1 and VEGF was detected in the same anatomical kidney compartments (glomerulus, arteries, and distal tubules). Renal function at the first week posttransplantation (analyzed by serum creatinine levels) showed a significant correlation with both HO-1 and VEGF mRNA expression, reinforcing the protective role of both genes in the early events of transplantation. It is concluded that the lower expression of HO-1, VEGF165, and Bcl-2 in cadaveric donor kidneys can reflect a defective adaptation against ischemia-reperfusion injury that may affect their function in the short term.

http://www.jasn.org/cgi/content/abstract/15/10/2548

Autosomal dominant polycystic kidney disease (ADPKD) is a commonly inherited disorder in humans that causes the formation of fluid-filled renal cysts, often leading to renal failure. PKD1 mutations cause 85% of ADPKD. Feline PKD is autosomal dominant and has clinical presentations similar to humans. PKD affects ~38% of Persian cats worldwide, which is ~6% of cats, making it the most prominent inherited feline disease. Previous analyses have shown significant linkage between the PKD phenotype and microsatellite markers linked to the feline homolog for PKD1. In this report, the feline PKD1 gene was scanned for causative mutations and a C>A transversion was identified at c.10063 (human ref NM_000296) in exon 29, resulting in a stop mutation at position 3284, which suggests a loss of ~25% of the C-terminus of the protein. The same mutation has not been identified in humans, although similar regions of the protein are truncated. The C>A transversion has been identified in the heterozygous state in 48 affected cats examined, including 41 Persians, a Siamese, and several other breeds that have been known to outcross with Persians. In addition, the mutation is segregating concordantly in all available PKD families. No unaffected cats have been identified with the mutation. No homozygous cats have been identified, supporting the suggestion that the mutation is embryonic lethal. These data suggest that the stop mutation causes feline PKD, providing a test to identify cats that will develop PKD and demonstrating that the domestic cat is an ideal model for human PKD.


http://www.jasn.org/cgi/content/abstract/13/5/1179

ABSTRACT. Abnormal traffic of proteins through the glomerular capillary has an intrinsic toxicity that results in tubular dysfunction and interstitial inflammation. It has been previously shown that in porcine proximal tubular cells high concentrations of albumin activated NF-κB, which is responsible for the enhanced synthesis of the inflammatory chemokine RANTES. This study investigates whether reactive oxygen species (ROS) served as second messengers in protein overload-induced NF-κB activation. Human proximal tubular cells (HK-2) were incubated (5 to 60 min) with human albumin and IgG (1 to 30 mg/ml). Both proteins induced a rapid or significant increase in hydrogen peroxide (H2O2) production at 5 min and persisting at 60 min. This effect was dose-dependent. The contribution of H2O2 in regulating NF-κB activation was evaluated by using the antioxidants dimethyl-thiourea and pyrrolidine dithiocarbamate in protein-overloaded HK-2 cells. Both agents, by preventing H2O2 generation, induced human albumin or IgG inhibited NF-κB activation. Stimulation of HK-2 with exogenous H2O2 resulted in the activation of a NF-κB subunit pattern similar to that obtained after protein challenge. Specific inhibitors of protein kinase C (PKC) activity significantly prevented H2O2 production and consequent NF-κB activation, suggesting that ROS generation in HK-2 cells occurs downstream of PKC activation. Either antioxidants or PKC inhibitor almost completely abolished the upregulation of the monocyte chemoattractant protein-1 gene induced by excess albumin, as evaluated by real-time PCR, thus supporting a role for PKC and ROS as critical signals for the expression of NF-κB-dependent inflammatory genes. To identify the enzymatic sources responsible for the increased H2O2 production, the effect of dyphenyleedioinonide, an inhibitor of the membrane NADP(H) oxidase, was studied, as was the effect of rotenone, which blocks complex I of the mitochondrial respiratory chain. It was found that both agents significantly reduced the exaggerated H2O2 induced by protein overload. These data
indicate that exposure to excess proteins in proximal tubular cells induces the formation of ROS, which are responsible for NF-{kappa}B activation and consequent induction of NF-{kappa}B-dependent inflammatory signals.


http://www.jasn.org/cgi/content/abstract/15/3/695

ABSTRACT. The anti-rat CD4 mAb RIB5/2 is very potent in inducing allospecific tolerance in vivo. It is interesting that the unresponsiveness is breakable by exogenous IL-2 applied during the induction phase of tolerance. The molecular mechanisms underlying anti-CD4 antibody-mediated inhibition of allospecific T cell activation and how this is antagonized by exogenous IL-2 were investigated. Anti-CD4 treatment, in vivo and in vitro, completely abrogated IL-2 production by alloreactive T cells. In contrast, anti-CD4-treated alloactivated T cells showed similar IFN-{gamma} mRNA expression as untreated alloactivated T cells but did not secrete any protein. Thus, the anti-CD4 antibody cannot prevent IFN-{gamma} mRNA expression but is interfering with posttranscriptional mechanisms that control IFN-{gamma} production during alloactivation of T cells. Addition of IL-2 but not IL-15 to anti-CD4-treated alloactivated T cells restored IFN-{gamma} protein production without leading to enhanced IFN-{gamma} mRNA expression. Further investigations revealed a diminished activation of translation initiation factor eIF2{alpha} in anti-CD4-treated T cells, which was restored by exogenous IL-2. As activated eIF2{alpha} is essential for the translation of IFN-{gamma} mRNA, the results may explain the reversibility of anti-CD4-induced unresponsiveness by exogenous IL-2. Furthermore, these results not only shed further light onto the molecular mechanisms of tolerance induction but also reveal the possible weaknesses of anti-CD4 antibody-induced unresponsiveness.


http://www.jasn.org/cgi/content/abstract/14/11/2958

ABSTRACT. For identifying potential diagnostic markers of proteinuric glomerulopathies, glomerular mRNA levels of molecules relevant for podocyte function (alpha-actinin-4, glomerular epithelial protein 1, Wilms tumor antigen 1, synaptopodin, dystroglycan, nephrin, podoplanin, and podocin) were determined by quantitative real-time RT-PCR from microdissected glomeruli. Biopsies from 83 patients with acquired proteinuric diseases were analyzed (minimal change disease [MCD; n = 13], benign nephrosclerosis [n = 16], membranous glomerulopathy [n = 31], focal and segmental glomerulosclerosis [FSGS; n = 9], and controls [n = 14]). Gene expression levels normalized to two different housekeeping transcripts (glyceraldehyde-3-phosphate-dehydrogenase and 18 S rRNA) did not allow a separation between proteinuric disease categories. However, a significant positive correlation between (alpha)-actinin-4, glomerular epithelial protein 1, synaptopodin, dystroglycan, Wilms tumor antigen 1, and nephrin was found in all analyzed glomeruli, whereas podocin mRNA expression did not correlate. Because varying amounts of housekeeper cDNA per glomerulus can confound expression ratios relevant for a subpopulation of cells, an "in silico" microdissection was performed using a podocyte-specific cDNA as a reference gene. Expression ratio of podocin to synaptopodin, the two genes with the most disparate expression, allowed a robust separation of FSGS from MCD and nephrosclerosis. Segregation of FSGS from MCD via this ratio was confirmed in an independent population of formaldehyde-fixed archival biopsies (MCD, n = 5; FSGS, n = 4) after glomerular laser capture microdissection. In addition, the expression marker was able to predict steroid responsiveness in diagnostically challenging cases of MCD versus FSGS (n = 6). As the above approach can be
performed as an add-on diagnostic tool, these molecular diagnostic parameters could give novel information for the management of proteinuric diseases. E-mail: kretzler@medpoli.med.uni-muenchen.de


http://www.jasn.org/cgi/content/abstract/15/11/2828

p-Aminohippurate (PAH) is the classical substrate used in the characterization of organic anion transport in renal proximal tubular cells. Although basolateral transporters for PAH uptake from blood into the cell have been well characterized, there is still little knowledge on the apical urinary efflux transporters. The multidrug resistance protein 2 (MRP2/ABCC2) is localized to the apical membrane and mediates ATP-dependent PAH transport, but its contribution to urinary PAH excretion is not known. In this report, we show that renal excretion of PAH in isolated perfused kidneys from wild-type and Mrp2-deficient (TR-) rats is not significantly different. Uptake of [14C]PAH in membrane vesicles expressing two different MRP2 clones isolated from Sf9 and MDCKII cells exhibited a low affinity for PAH (Sf9, 5 +/- 2 mM; MDCKII, 2.1 +/- 0.6 mM). Human MRP4 (ABCC4), which has recently been localized to the apical membrane, expressed in Sf9 cells had a much higher affinity for PAH (Km = 160 +/- 50 {micro}M). Various inhibitors of MRP2-mediated PAH transport also inhibited MRP4. Probenecid stimulated MRP2 at low concentrations but had no effect on MRP4; but at high probenecid concentrations, both MRP2 and MRP4 were inhibited. Sulfinpyrazone only stimulated MRP2, but inhibited MRP4. Real-time PCR and Western blot analysis showed that renal cortical expression of MRP4 is approximately fivefold higher as compared with MRP2. MRP4 is a novel PAH transporter that has higher affinity for PAH and is expressed more highly in kidney than MRP2, and may therefore be more important in renal PAH excretion.


http://www.jasn.org/cgi/content/abstract/14/2/338

ABSTRACT. The p38 mitogen-activated protein kinase (MAPK) pathway is a pro-inflammatory signal transduction pathway. The aim of this study was to examine the role of this pathway in acute renal inflammation. Immunostaining localized components of the p38 MAPK pathway (p38(alpha), p-p38, p-ATF-2) in normal glomeruli, to podocytes, and occasional endothelial cells. This study identified an eightfold increase in glomerular activation of p38 MAPK (phosphorylated p38, p-p38) within 3 h of the induction of rat anti-glomerular basement membrane (GBM) glomerulonephritis and localized p-p38 and p-ATF-2 to infiltrating neutrophils, with increased staining of podocytes and endothelial cells. The relevance of these findings to human acute inflammatory renal disease was determined by examination of biopsy specimens. In patients with post-infectious glomerulonephritis, there was an increased number of positive p-p38 glomerular cells, including p-p38 staining of infiltrating neutrophils, compared with normal human kidney. In rats, administration of a specific p38 MAPK inhibitor, NPC 31145, before induction of anti-GBM disease prevented a loss of renal function and substantially reduced proteinuria. The reduction in renal injury was attributed to a 55% reduction in glomerular neutrophil infiltration and a 68% reduction in platelet accumulation. This was associated with an abrogation of glomerular P-selectin immunostaining and inhibition of glomerular P-selectin gene expression. In summary, this study has localized the components of the p38 MAPK pathway to cells in normal and diseased rat and human kidney and identified a number of important mechanisms by which signaling through
the p38 MAPK pathway induces inflammatory renal disease. Blockade of the p38 pathway may be a novel therapeutic strategy for the treatment of acute renal inflammation. E-mail: cosimo.stambe@med.monash.edu.au


http://www.jasn.org/cgi/content/abstract/13/11/2619

ABSTRACT. Transepithelial chloride and fluid secretion by many types of epithelia involves activation of a conductive K+ pathway that serves to support the electrochemical driving force for Cl- secretion. This study sought to determine if such a pathway is involved in Cl- and fluid secretion by the cystic epithelium in autosomal dominant polycystic kidney disease (ADPKD). Primary cultures of cells derived from the cysts of patients with ADPKD were used. Confluent monolayers of these cells, mounted in Ussing chambers, were stimulated to secrete Cl- by application of the adenyl cyclase agonist, forskolin. The effects of various K+ channel blockers on the increase in short-circuit current (Isc) generated by active Cl- secretion were determined. Charybdotoxin, an inhibitor of Ca2+-sensitive K+ channels exerted no effect. Similarly, the chromanole 293B, an inhibitor of cAMP-induced K+ conductance, exerted no effect on cAMP-dependent anion secretion. Glibenclamide, an inhibitor of ATP-sensitive K+ channels and the cystic fibrosis transmembrane conductance regulator (CFTR), modestly inhibited the forskolin-stimulated current when applied to the apical surface of the monolayers, suggesting a relatively weak effect on CFTR. Basolateral application of glibenclamide inhibited Isc to a greater extent. This latter effect may be due to inhibition of a K+-conductive transport step. Glibenclamide exerted little effect on the Isc of nonstimulated monolayers. Cyst growth in ADPKD is driven by cell proliferation and Cl- and fluid secretion. The effect of glibenclamide on the growth of cysts formed within a collagen gel by cultured ADPKD cells was tested. Addition of glibenclamide to the media bathing the cysts inhibited their growth. Glibenclamide also blocked the formation of cysts when it was added to the media at the time the cells were seeded within the collagen gel. Glibenclamide was also found to inhibit the proliferation of ADPKD cells. RT-PCR analysis demonstrated that the ATP-sensitive K+ channel, Kir 6.2, is expressed in cultured ADPKD cells and in normal human kidney. These results suggest that ATP-sensitive K+ channel blockers should be investigated as possible therapeutic agents to inhibit cyst growth in ADPKD.


http://www.jasn.org/cgi/content/abstract/15/9/2383

ABSTRACT. Myofibroblasts are pivotal participants in pathologic processes in a wide variety of organs, such as lung, liver, and kidney, by producing several inflammatory cytokines and extracellular matrices. The mechanism by which transdifferentiation from original cell to myofibroblast occurs, however, is still unclear. The expression of smooth muscle {alpha}-actin (SM{alpha}A) is the most characteristic feature of myofibroblasts; therefore, it was speculated that any factors that promote SM{alpha}A expression might be the key to transdifferentiation to myofibroblasts and disease exacerbation. A transcription factor CCAAT/enhancer-binding protein {delta} (C/EBP{delta}) was identified and demonstrated to bind to sequences including the CArG motif from SM{alpha}A intron 1 and to increase transcriptional activity of this promoter. Expression of SM{alpha}A and C/EBP{delta} in the glomerular area was upregulated in rat anti-Thy1 glomerulonephritis and mouse Habu-venom glomerulonephritis, both of which are models of mesangio-proliferative glomerulonephritis. In the latter model, C/EBP{delta} knockout mice...
demonstrated significantly less SM{alpha}A expression in the glomerular area on day 8 and less renal functional deterioration on day 14, compared with wild-type mice. These data suggest an important role for C/EBP(delta) in myofibroblast transdifferentiation and glomerulonephritis exacerbation.


http://www.jasn.org/cgi/content/abstract/13/10/2488

ABSTRACT. Platelets are thought to play an important role in the initiation and the progression of a variety of glomerulonephritides. This study examined whether platelets induce production of monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in leukocyte recruitment and glomerular injury, by cultured human mesangial cells (MC). To this end, platelets isolated from normal human donors were cocultured with MC at various ratios. MCP-1 synthesis was evaluated by quantitative real-time PCR and enzyme-linked immunosorbent assay. Platelets at 1:100 ratio (MC to platelets) induced an approximately 20-fold increase in mesangial MCP-1 mRNA and protein expression through an obligatory cell-to-cell contact-dependent mechanism. Importantly, blockade of the CD40/CD40 ligand (CD40L) pathway with neutralizing antibodies decreased MCP-1 production by approximately 60%. It was confirmed that CD40 was functionally expressed on MC. Gel-shift assays and inhibitors of phosphorylation were used to demonstrate that activation of p38 mitogen-activated protein kinase, protein tyrosine kinases, and nuclear factor-{kappa}B activation were essential for MCP-1 production. These data indicate that platelet/MC contact stimulates the production of MCP-1 and may contribute to glomerular inflammatory responses by recruiting leukocytes from the peripheral blood. E-mail: tkuroiwa@med.gunma-u.ac.jp


http://www.jasn.org/cgi/content/abstract/15/3/635

ABSTRACT. Hyperoxaluria leads to calcium oxalate (CaOx) crystallization and development of tubulointerstitial lesions in the kidneys. Treatment of hyperoxaluric rats with angiotensin II (Ang II) type I receptor blocker (ARB) reduces lesion formation. Because Ang II mediates osteopontin (OPN) synthesis, which is involved in both macrophage recruitment and CaOx crystallization, it was hypothesized that ARB acts via OPN. Hyperoxaluria was induced in 10-wk-old male Sprague-Dawley rats, and they were treated with ARB candesartan. At the end of 4 wk, kidneys were examined for crystal deposits, ED-1-positive cells, and expression of OPN mRNA. PCR was used to quantify OPN, renin, and angiotensin-converting enzyme (ACE) mRNA in kidneys. RIA was used to determine renal, plasma, and urinary OPN; plasma renin; Ang II and ACE; and renal Ang II. For evaluating oxidative stress, malondialdehyde was measured. Urinary calcium, oxalate, creatinine, and albumin were also determined. Despite similar urinary calcium and oxalate levels, kidneys of hyperoxaluric rats on candesartan had fewer CaOx crystals, fewer ED-1-positive cells, reduced OPN expression, and reduced malondialdehyde than hyperoxaluric rats. Urinary albumin excretion and serum creatinine levels improved significantly on candesartan treatment. mRNA for OPN, renin, and ACE were significantly elevated in hyperoxaluric rats. OPN synthesis and production increased with hyperoxaluria but to a lesser extent in candesartan-treated hyperoxaluric rats. These results show for the first time that oxalate can activate the renal renin-angiotensin system and that oxalate-induced upregulation of OPN is in part mediated via renal
Connective tissue growth factor (CTGF) is implicated as a factor promoting tissue fibrosis in several disorders, including diabetic nephropathy. However, the molecular mechanism(s) by which it functions is not known. CTGF rapidly activates several intracellular signaling molecules in human mesangial cells (HMC), including extracellular signal-related kinase 1/2, Jun NH2-terminal kinase, protein kinase B, CaMK II, protein kinase C(α), and protein kinase C(δ), suggesting that it functions via a signaling receptor. Treating HMC with CTGF stimulated tyrosine phosphorylation of proteins 75 to 80 and 140 to 180 kD within 10 min, and Western blot analysis of anti-phosphotyrosine immunoprecipitates identified the neurotrophin receptor TrkA (molecular weight approximately 140 kD). Cross-linking rCTGF to cell surface proteins with 3,3'-dithiobis(sulfosuccinimidylpropionate) revealed that complexes formed with TrkA and with the general neurotrophin co-receptor p75NTR. rCTGF stimulated phosphorylation of TrkA (tyr 490, 674/675). K252a, a known selective inhibitor of Trk, blocked this phosphorylation, CTGF-induced activation of signaling proteins, and CTGF-dependent induction of the transcription factor TGF-β-inducible early gene in HMC. It is concluded that TrkA serves as a tyrosine kinase receptor for CTGF.

J. Antimicrob. Chemother. (13)


Objectives: To assess the relationship between the presence of DHFR and DHPS mutations in Plasmodium falciparum, parasite in vitro resistance, and in vivo efficacy of sulfadoxine-pyrimethamine (SP) treatment. Patients and methods: Measurement of SP treatment efficacy in malaria-infected children in Gabon was combined with in vitro tests of susceptibility to pyrimethamine and cycloguanil, and molecular genotyping at several DHFR and DHPS loci of parasites isolated before treatment. DHFR was studied at codons 108, 51, and 59, whereas DHPS gene was typed at positions 436, 437, 540 and 581. Results: SP treatment was effective in 86% of children by day 28. Seventy-five percent of isolates were in vitro resistant to pyrimethamine and 65.5% to cycloguanil. No mutation was detected at codons 540 and 581 of the DHPS gene. Most isolates (71.8%) presented with the triple mutant DHFR genotype, whereas 64.3% combined at least three DHFR and one DHPS mutations. The increase in the number of DHFR mutations was associated with an increase in in vitro resistance to pyrimethamine and cycloguanil; three DHFR mutations conferred pyrimethamine and to a lesser extent cycloguanil resistance. Treatment failures only occurred with isolates presenting at least two DHFR mutations (S108N and C59R) and one DHPS mutation (S436A or A437G), but SP treatment of infections with such parasites gave treatment success in 82.0% of children. Conclusions: DHFR mutations
that lead to high-level in vitro resistance to pyrimethamine plus 1-2 DHPS mutations are not sufficient to induce in vivo failure of SP treatment in young children from Gabon.


http://jac.oupjournals.org/cgi/content/abstract/54/3/680

Objectives: To genotypically characterize the vancomycin resistance mechanism of Enterococcus faecium N03-0072, which was negative by PCR for the currently known van genotypes. Methods: PCR was used to amplify the entire vancomycin resistance operon and the complete nucleotide sequence was determined by dideoxy cycle sequencing. Results: Analysis revealed a VanD-type operon with 94% nucleotide identity to the VanD4 operon and 90% nucleotide identity to the VanD1/D3 operons. A set of universal primers was designed in order to identify all current vanD variants by PCR. Conclusions: E. faecium N03-0072 carries a new VanD-type operon, designated VanD5.


http://jac.oupjournals.org/cgi/content/abstract/55/4/506

Objectives: To evaluate the usefulness of cefoxitin when used as a surrogate marker for the detection of methicillin resistance. Patients and methods: Eight hundred and seventy-one strains of Staphylococcus aureus, collected from eight tertiary referral centres serving diverse socio-economic populations, were included in the study using NCCLS disc diffusion and the agar dilution methods. Results: Using cefoxitin and NCCLS criteria for disc diffusion, the sensitivity and specificity for recognizing methicillin resistance were both 100%. Similar results were obtained when the strains were tested by the agar dilution method. The cefoxitin MICs for methicillin-susceptible strains were [\le] 4 mg/L. Conclusions: Testing with cefoxitin as a surrogate marker for the detection of methicillin resistance was very accurate with both disc diffusion and agar dilution methods. Such testing clearly distinguished methicillin-resistant strains of S. aureus from methicillin-susceptible strains.


http://jac.oupjournals.org/cgi/content/abstract/55/1/123

Objectives: The impact of agricultural use of antimicrobials on the present and future efficacy of therapeutic drugs in human medicine is a growing public concern. Quinupristin/dalfopristin has been approved to treat human disease caused by vancomycin-resistant Enterococcus faecium and is related to virginiamycin, a streptogramin complex that has long been used in USA agriculture poultry production. Methods: Streptogramin-resistant isolates of E. faecium from poultry production environments on the eastern seaboard were recovered without selection for streptogramin resistance and examined using ribotyping to evaluate clonal bias. Colony PCR
screening for the previously described streptogramin resistance determinants erm(A), erm(B), msr(C), vgb(A), vat(D) and vat(E) was performed to determine the prevalence of streptogramin resistance mechanisms from these environments. Results: The collection of E. faecium isolates was unevenly distributed among 28 ribogroups and did not cluster geographically. The most prevalent ribogroups was composed of isolates that possessed diverse antimicrobial resistance profiles. Of the 127 isolates examined, 63% were resistant to quinupristin/dalfopristin. The resistance determinants erm(A) and erm(B) were observed among 6% and 10%, respectively, of streptogramin-resistant isolates. msr(C) was detected in a single isolate that was resistant to macrolide and lincosamide antibiotics. The streptogramin B hydrolase vgb(A) and the streptogramin A acetyltransferases genes vat(D) and vat(E) were not detected in any of the E. faecium isolates. Conclusions: These results indicate that there is widespread resistance to streptogramin antimicrobials among E. faecium throughout the poultry production region in this study and that the mechanisms of resistance to streptogramin antimicrobials within this population remain largely uncharacterized.


http://jac.oupjournals.org


http://jac.oupjournals.org/cgi/content/abstract/53/6/989

Objective: This study compared the attached biofilm populations on acrylonitrile-butadiene-styrene (ABS) plastic with and without the incorporation of the antimicrobial triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] after 1-3 weeks of exposure to drinking water. Methods: Biofilms were cultivated on triclosan-incorporated (TP) and control plastics (CP) in continuous flow culture reactors with drinking water as the growth medium and inoculum. After 1-3 weeks of exposure, the plastics were removed and the biofilms aseptically harvested. The attached communities were examined with respect to direct cell counts, culturability, triclosan resistance and community composition. Results: Based on these analyses, no significant differences were observed between the populations attached to TP and CP surfaces. Results from both a bioavailability assay and gas chromatography mass spectrometry analyses, revealed that only trace amounts of triclosan desorbed from the plastic. The lack of biofilm community difference, coupled with this limited desorption of triclosan from the TP indicates that the ABS plastic studied was no more effective at controlling bacterial populations than the control plastic because the antimicrobial was not bioavailable. Conclusions: These results call into question the long-term utility of triclosan incorporation into ABS plastic and highlight the need for proof of efficacy regarding the antimicrobial properties of such materials.


http://jac.oupjournals.org/cgi/content/abstract/50/4/513

Antibiotic resistance of urinary tract pathogens has increased worldwide. Our aim was to provide
information regarding resistance patterns of Escherichia coli in urinary tract infections (UTIs) and E. coli bacteraemia in Denmark. The overall resistance ranged from: ampicillin 20-47%, mecillinam 0-7%, trimethoprim 10-28%, sulfamethizole 22-47% and nitrofurantoin 0-3%. In strains with sulfamethizole MICs > 2048 mg/L, 97% carried sull, sull or both genes, with sull being the most common. Among the sull gene-positive strains, 96% were int1 gene positive.


http://jac.oupjournals.org/cgi/content/abstract/55/1/22

Objectives: Quinolone resistance in the opportunistic pathogen Pseudomonas aeruginosa is commonly caused by mutations that alter the target molecules DNA gyrase/topoisomerase IV, or cause activation of various efflux systems. We have analysed the effect of quinolone resistance caused by DNA gyrase/topoisomerase IV mutations on bacterial fitness. Methods: Norfloxacin-resistant mutants were isolated and by DNA sequencing the mutations conferring resistance were identified. Mutant fitness was determined by measuring growth rates in vitro. Mutants with reduced growth rates were serially passaged to obtain growth-compensated mutants. The level of DNA supercoiling was determined by isolating plasmid DNA from the susceptible, resistant and compensated mutants and comparing the topoisomer distribution patterns by gel electrophoresis in the presence of chloroquine. Results: Low-level resistance (4-48 mg/L) was caused by single mutations in gyrA or gyrB. Among these strains, three out of eight mutants showed lower fitness, whereas high-level resistant (>256 mg/L) mutants with double mutations in gyrA and parC, parE, nfxB or unknown genes all showed a reduced fitness. Slow-growing resistant mutants with a gyrA mutation had decreased DNA supercoiling. After serial passage in laboratory medium, mutant fitness was increased by compensatory mutation(s) that restored supercoiling to normal levels. The compensatory mutation(s) was not located in any of the genes (gyrAB, topA, parCE, hupB, fis, hupN, himAD or PA5348) that were expected to affect supercoiling. Conclusions: Our results show that no cost and compensatory mutations are common in quinolone-resistant P. aeruginosa.


http://jac.oupjournals.org/cgi/content/abstract/55/5/655

Objectives: The aim of this study was to identify changes in the gene expression profile of Candida albicans upon exposure to the hydroxypyridone anti-infective agent ciclopirox olamine in an effort to better understand its mechanism of action. Methods: C. albicans SC5314 was exposed to either medium alone or ciclopirox olamine at a concentration equivalent to the IC50 (0.24 mg/L) for 3 h. RNA was isolated and gene expression profiles were compared using DNA microarrays. Differential expression of select genes was confirmed by real-time reverse transcription (RT)-PCR. Mutants disrupted for CDR2 and both CDR1 and CDR2, as well as a clinical isolate overexpressing CDR1 and CDR2, were examined for changes in susceptibility to ciclopirox olamine. Results: A total of 49 genes were found to be responsive to ciclopirox olamine, including 36 up-regulated genes and 13 down-regulated genes. These included genes involved in small molecule transport (HGT11, HXT5, ENA22, PHO84, CDR4), iron uptake (FRE30, FET34, FTR1, FTR2, SIT1) and cell stress (SOD1, SOD2, CDR1, DDR48). Mutants disrupted for CDR2 and both CDR1 and CDR2, as well as a clinical isolate overexpressing CDR1 and CDR2, showed no change in susceptibility to ciclopirox olamine compared with the respective parent. Conclusions: Consistent with the hypothesis that ciclopirox olamine acts as an iron chelator, it induced changes in expression of many genes involved in iron uptake. Despite induction of the
multidrug efflux pump genes CDR1 and, to a lesser extent, CDR2 by ciclopirox olamine, these genes do not affect susceptibility to this agent.


http://jac.oupjournals.org/cgi/content/abstract/49/5/793

Ninety clinical Staphylococcus aureus isolates from separate patients were examined phenotypically and genotypically for susceptibility to methicillin/oxacillin. Thirty were methicillin/oxacillin susceptible and 60 were methicillin and oxacillin resistant (MRSA). The 60 MRSA isolates examined were subdivided into two groups according to their antibiotic profiles and comprised 30 non-multidrug-resistant (NMDR) isolates, resistant to less than two non-\(\beta\)-lactam antibiotics, and 30 multidrug-resistant (MDR) isolates, resistant to three or more non-\(\beta\)-lactam antibiotics. Phenotypic and genotypic analysis of methicillin/oxacillin showed that despite use of the guidelines published by the NCCLS for the testing of S. aureus susceptibility to methicillin/oxacillin, MIC values of some NMDR MRSA isolates fell below the NCCLS-recommended breakpoints. Etest strips failed to detect two NMDR MRSA isolates tested with oxacillin and four tested with methicillin. Lowering the NCCLS-recommended oxacillin screen agar concentration from 6 to 2 mg/L and temperature of incubation to 30\(\degree\)C, improved the specificity and sensitivity of NMDR MRSA detection from 87\% to 100\%. On PFGE analysis these NMDR MRSA strains were genotypically different. Genotypic tests, such as multiplex PCR for the mecA/nuc genes and DNA hybridization for the mecA gene, or phenotypic monoclonal antibody-based tests to detect penicillin-binding protein 2a (PBP2a) offer advantages for problematic isolates in detecting or confirming low-level phenotypic heterogeneous mecA expression of oxacillin and methicillin resistance in NMDR MRSA.


http://jac.oupjournals.org/cgi/content/abstract/51/4/787

We have mapped the variable region of the two class 1 integrons found in the multiresistant strain \textit{Providencia stuartii} 1723. Integron 1 contains a new arrangement of gene cassettes, aacA4-aadB-aadA1, conferring resistance to all aminoglycosides used for clinical treatment. Integron 2 contains a variant of the gene cassette \textit{ere(A)}, coding for an erythromycin esterase, whose nucleotide sequence shares 93.7\% DNA identity with \textit{ere(A)} from \textit{Escherichia coli} BM2195 plasmid pIP1100.


http://jac.oupjournals.org/cgi/content/abstract/51/2/419

The EVIGENE MRSA Detection Kit was evaluated on coagulase-negative staphylococci (CoNS) from agar plates and on staphylococci directly from positive spiked blood cultures. For the CoNS study, a total of 242 isolates were tested, and of these 237 gave valid test results. For the 237
valid tests, all gave correct mecA classification. For the blood culture procedure, a collection of 51 mecA-positive Staphylococcus aureus, 21 mecA-negative S. aureus, 31 mecA-positive CoNS and 28 mecA-negative CoNS were used for the simulated blood cultures. For the S. aureus strains, all gave valid test results and correct mecA classification. One of the MRSA isolates gave a very faint nuc signal, and another four isolates gave results close to the cut-off of the kit; however, these were still clearly positive when read by the naked eye. For the CoNS isolates, 51 of the 59 strains gave valid results. All of these 51 strains gave correct mecA status. Thus the EVIGENE MRSA Detection Kit can provide fast and accurate determination of methicillin resistance in CoNS. This preliminary study of the blood culture procedure indicates that it is possible to achieve determination of methicillin resistance in staphylococci 8 h after positivity of the blood culture, making same-day detection of methicillin resistance possible.


http://jac.oupjournals.org/cgi/content/abstract/54/6/1007

Objectives: This study was conducted to investigate the occurrence of 16S rRNA methylases that confer high-level aminoglycoside resistance in Klebsiella pneumoniae and Escherichia coli isolates from two Taiwanese hospitals and the characteristics of these isolates. Methods: A total of 1624 K. pneumoniae and 2559 E. coli isolates consecutively collected over an 18 month period from a university hospital and seven E. coli and eight K. pneumoniae isolates that were resistant to amikacin from a district hospital were analysed. Two 16S rRNA methylase genes, armA and rmtB, were detected by PCR-based assays. {beta}-Lactamase characteristics were determined by phenotypic and genotypic methods. Results: Overall, 28 armA-positive and seven rmtB-positive isolates were identified, and extended-spectrum {beta}-lactamas (ESBLs) were detected in 33 (94.3%) isolates. The prevalence rates of armA and rmtB at the university hospital were 0.9% (n=15) and 0.3% (n=5) in K. pneumoniae and 0.4% (n=10) and 0.04% (n=1) in E. coli. CTX-M-3, CTX-M-14, SHV-5-like ESBLs, and CMY-2 were detected alone or in combination in 21, 6, 11, and 2, respectively, of the 28 armA-positive isolates. CTX-M-14 was detected in six of the seven rmtB-positive isolates. Fingerprinting of conjugative plasmids revealed the dissemination of closely related plasmids containing both armA and blaCTX-M-3. PFGE suggests that armA and rmtB spread by both horizontal transfer and clonal spread. Conclusions: This is the first report of the emergence of 16S rRNA methylases in Enterobacteriaceae in Taiwan. The spread of the multidrug-resistant isolates producing both ESBLs and 16S rRNA methylases may become a clinical problem.

J. Biochem. (Tokyo) 133(6): 825-831.


http://jb.oupjournals.org/cgi/content/abstract/133/6/825

Two cultured cell lines, called Kan-R1 and Kan-R2, were established from rat hepatic cells by in vitro culture with a hepatocarcinogen, 3-methoxy-4-aminoazobenzene, and examined for the
gene expression of cytochrome P450 (P450) isoforms, CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1 and CYP3A2, by the RT-PCR method. It was revealed that all the P450 genes examined were expressed in both cell lines, although the two cell lines differed in cell size and colony-forming ability on a soft agar. The expression levels of the CYP1A2, CYP2B1, CYP2B2, CYP3A1, and CYP3A2 genes were lower than those in liver tissues, while that of CYP1A1 was higher in the cell lines. In both cell lines, cycloheximide, an inhibitor of protein synthesis, augmented the gene expression of the P450s except CYP2B1. These findings indicate that the newly established hepatic cell lines substantially express the P450 genes for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1, and CYP3A2, and that the constitutive gene expression of these P450s, with the exception of CYP2B1, may be inhibited by negative transcription factors.


http://jb.oupjournals.org/cgi/content/abstract/136/2/211

The side-chain asymmetry of physiological porphyrins is produced by the cooperative action of hydroxymethylbilane synthase and uroporphyrinogen (uro'gen) III synthase. Although the role of uro'gen III synthase is essential for the chemistry of porphyrin biosynthesis, many aspects, structural as well as mechanical, of uro'gen III synthase have yet to be studied. We report here an expression system in Escherichia coli and a purification procedure for human uro'gen III synthase. The enzyme in the lysate was unstable, but we found that glycerol prevents the activity loss in the lysate. The purified enzyme showed remarkable thermostability, particularly when kept in phosphate buffer containing DTT or EDTA, indicating that the enzyme activity may depend on its oxidation state. Examination of the relationship between the number of Cys residues that are accessible to 5,5'-dithiobis(2-nitrobenzoic acid) and the remaining activity during heat inactivation showed that a particular Cys residue is involved in activity loss. From the crystal structure of human uro'gen III synthase [Mathews et al. (2001) EMBO J. 20, 5832-5839], this Cys residue was considered to be Cys73, which is buried deep inside the enzyme, suggesting that Cys73 of human uro'gen III synthase plays an important role in enzyme activity.


http://jb.oupjournals.org/cgi/content/abstract/134/1/143

The gene coding for microphthalmia-associated transcription factor (Mitf) contains many promoters that could generate multiple Mitf isoforms with distinct amino-termini, such as ubiquitously expressed Mitf-A and Mitf-H. To gain further insight into Mitf isoform multiplicity and the regulation of the promoter usage of the Mitf gene, we have analyzed the function of the amino-terminal domains of Mitf isoforms and the expression of Mitf mRNA in mouse postnatal testis, which is characterized by spermatogenesis and a cool temperature because of its unique location. Here we show that the amino-terminal domain of Mitf-A possesses a transactivation activity, as judged by yeast expression analysis. We also show the expression of Mitf-A and Mitf-D mRNAs in testis by PCR-based methods. Moreover, in situ hybridization analysis revealed that an Mitf mRNA, probably representing Mitf-A and/or Mitf-D, is expressed in germ cells, including spermatogonia, spermatocytes that undergo meiosis, and round spermatids with the haploid genome, but is undetectable in elongated spermatids with remodeled and condensed chromatin. Notably, Mitf mRNA is undetectable in somatic Leydig cells and peritubular cells. Therefore, multiple promoters may direct differential expression of the Mitf gene in the testis and contribute to functional diversity of Mitf isoforms.
A procedure was established for expression and purification of abundant recombinant cold-active protein-tyrosine-phosphatase (RCPTPase), which showed identical enzymatic characteristics to the native enzyme (NCPTPase). The purified RCPTPase showed high catalytic activity at low temperature and maximal activity at 30{degrees}C. RCPTPase has a thermodynamic characteristic in that its activation enthalpy was determined to be low, 4.3 kcal/mol, at temperatures below 19.3{degrees}C, where the Arrhenius relationship exhibited an inflection point, in comparison with 20.3 kcal/mol above 19.3{degrees}C. Also, the thermostability, \( \Delta G_{\text{water}} \), of the catalytic site in the RCPTPase molecule was increased with a decrease in temperature. It was considered that cold-active protein-tyrosine-phosphatase could maintain its catalytic site in a stable conformation for eliciting high catalytic activity with low activation enthalpy at low temperature.


The presence of several copies of the same class of repetitive element in DNA templates increases the probability of ambiguous base calling caused by band compression artifacts in the BigDye (Applied Biosystems, Foster City, CA) terminator cycle sequencing method. The presence of di-, tri-, and tetranucleotide repeats and short tandem repeats, which is widespread in the genome, poses a daunting task in sequencing laboratories, where a variety of DNA templates are submitted for sequencing. These base anomalies arise mainly as a result of the formation of secondary structures, including hairpins, and intramolecular base pairing between guanine and cytosine bases on the template strand. A common approach to the optimization of such sequencing reactions is either to replace the guanine with a base analog (such as deoxyinosine 5'-triphosphate [dITP] or 7-deaza-deoxyguanosine 5'-triphosphate [dGTP]) or to add a denaturant (such as dimethylsulfoxide [DMSO]) to the reaction mixture to overcome the undesired sequencing artifacts. Additives sometimes are ineffective for sequencing templates with GC-rich regions and repeat sequences. Herein we describe the effectiveness of (carboxymethyl)trimethylammonium (betaine) inner salt as an additive in the reaction mixture for reducing band compressions. The results presented show that betaine outperformed DMSO in sequencing through the localized regions containing GC-rich base pairs, guanine stretches, or TGC-type repeats in several DNA templates.

With the advent of high-density DNA marker data sets for the mouse and other model systems, 100 or more genotypes are routinely generated from large groups of mice. Issues of the accuracy and reliability of the genotyping are extremely important but often not addressed until genetic analysis is conducted. Simple tests that rely on the robust predictions arising from Mendelian genetics can be made quickly in the molecular laboratory as the data are generated, and require only a spreadsheet program. In this report, genotype data from 392 mice tested at 96 marker sites were analyzed for errors that are typical when handling large volumes of data generated in a repetitive process. The testing consisted of: (1) repeating the genotyping of approximately 1% of the samples; (2) examining the deviation from the expected segregation ratio (1:2:1) on a marker-by-marker basis; and (3) testing the correlation of the genotype at one marker with that at neighboring genetic markers on a chromosome. These three steps allowed analysis at the level of the microtiter plate, where errors are most likely to occur. A set of 96 dinucleotide repeat markers that are polymorphic between the C57BL/6J and DBA/2J mouse strains and can be multiplexed is reported for use in other genotyping projects.


DNA sequencing core facilities serve as centralized resources within both academic and commercial institutions, providing expertise in the area of DNA analysis. The composition and configuration of these facilities continue to evolve in response to new developments in instrumentation and methodology. The goal of the 2003 DNA Sequencing Research Group (DSRG) survey was to identify recent changes in staffing, funding, instrumentation, services, and customer relations. Responses to 58 survey questions from 30 participants are presented to offer a look at the current typical DNA core sequencing facility. The results from this study will serve as a resource for institutions to benchmark their shared core laboratories, and to give facility directors an opportunity to compare and contrast their respective services and experiences.

J. Bone Joint Surg. Am. (1)


Background: Periprosthetic tissue formation and local inflammation that are associated with wear debris contribute to the pathogenesis of aseptic loosening of a prosthesis. This study evaluated a retrovirus-mediated gene therapy with use of a novel xenograft-based animal model. Methods: Human periprosthetic tissues obtained from patients during revision arthroplasty performed because of aseptic loosening of a prosthetic joint were transplanted into the left quadriceps and paravertebral muscles of severe combined immunodeficient (SCID) mice. The engrafted tissues
were recovered seven, fifteen, or thirty days after implantation for histological and molecular analyses. The periprosthetic tissues were incubated with retroviruses encoding for human interleukin-1 receptor antagonist (hIL-1Ra) or bacteria β-galactosidase (LacZ) at 37°C for three hours prior to implantation to evaluate their responses to gene modification. Results: The human periprosthetic tissues were well accepted in SCID mice for up to thirty days, with angiogenesis occurring in the majority of the implanted tissue sections. The histological appearance was consistent between the recovered graft tissue and the original donor tissue. Strong expression of interleukin-1, tumor necrosis factor, and interleukin-6 was detected in the xenografts with use of immunohistochemical stains. Histological analysis revealed that interleukin-1 receptor antagonist gene modification significantly decreased the total number of inflammatory cells (p < 0.01) in engrafted human tissue containing implant wear debris. Real-time reverse transcription-polymerase chain reaction and immunohistochemical staining showed declining expression levels of interleukin-1 and tumor necrosis factor following interleukin-1 receptor antagonist gene transfer in comparison with LacZ-transduced or virus-free controls.

Conclusions: Human periprosthetic tissue can survive in the SCID mouse host for up to thirty days and responds to the interleukin-1 receptor antagonist gene transfer with the amelioration of inflammation. Clinical Relevance: The human periprosthetic tissue-SCID mouse chimera has been characterized in this study as a useful model to explore the properties of human periprosthetic tissue in vivo, laying the foundation for potential clinical application of gene therapy in aseptic loosening.

J. Cell Biol. (2)


http://www.jcb.org/cgi/content/abstract/167/1/123

Disruption of latent TGF-(β) binding protein (LTBP)-4 expression in the mouse leads to abnormal lung development and colorectal cancer. Lung fibroblasts from these mice produced decreased amounts of active TGF-(β), whereas secretion of latent TGF-(β) was significantly increased. Expression and secretion of TGF-(β)2 and -(β)3 increased considerably. These results suggested that TGF-(β) activation but not secretion would be severely impaired in LTBP-4-/- fibroblasts. Microarrays revealed increased expression of bone morphogenic protein (BMP)-4 and decreased expression of its inhibitor gremlin. This finding was accompanied by enhanced expression of BMP-4 target genes, inhibitors of differentiation 1 and 2, and increased deposition of fibronectin-rich extracellular matrix. Accordingly, increased expression of BMP-4 and decreased expression of gremlin were observed in mouse lung. Transfection of LTBP-4 rescued the -/- fibroblast phenotype, while LTBP-1 was inefficient. Treatment with active TGF-(β)1 rescued BMP-4 and gremlin expression to wild-type levels. Our results indicate that the lack of LTBP-4-mediated targeting and activation of TGF-(β)1 leads to enhanced BMP-4 signaling in mouse lung.


http://www.jcb.org/cgi/content/abstract/156/6/1077
The molecular mechanisms of apoptosis are highly conserved throughout evolution. The homologs of genes essential for apoptosis in Caenorhabditis elegans and Drosophila melanogaster have been shown to be important for apoptosis in mammalian systems. Although a homologue for CED-4/apoptotic protease-activating factor (Apaf)-1 has been described in Drosophila, its exact function and the role of the mitochondrial pathway in its activation remain unclear. Here, we used the technique of RNA interference to dissect apoptotic signaling pathways in Drosophila cells. Inhibition of the Drosophila CED-4/Apaf-1-related killer (ARK) homologue resulted in pronounced inhibition of stress-induced apoptosis, whereas loss of ARK did not protect the cells from Reaper- or Grim-induced cell death. Reduction of DIAP1 induced rapid apoptosis in these cells, whereas the inhibition of DIAP2 expression did not but resulted in increased sensitivity to stress-induced apoptosis; apoptosis in both cases was prevented by inhibition of ARK expression. Cells in which cytochrome c expression was decreased underwent apoptosis induced by stress stimuli, Reaper or Grim. These results demonstrate the central role of ARK in stress-induced apoptosis, which appears to act independently of cytochrome c. Apoptosis induced by Reaper or Grim can proceed via a distinct pathway, independent of ARK.


http://jcs.biologists.org/cgi/content/abstract/117/2/243

Rac2 is a Rho GTPase that is expressed in cells of hematopoietic origin, including neutrophils and macrophages. We recently described an immunodeficient patient with severe, recurrent bacterial infections that had a point mutation in one allele of the Rac2 gene, resulting in the substitution of aspartate 57 with asparagine. To ascertain further the effects of Rac2D57N in leukocytes, Rac2D57N was expressed in primary murine bone-marrow-derived macrophages (cells that we show express approximately equal amounts of Rac1 and Rac2). Rac2D57N expression in macrophages inhibited membrane ruffling. Rac2D57N expression inhibited the formation of macropinosomes, demonstrating a functional effect of the loss of surface membrane dynamics. Surprisingly, Rac2D57N induced an elongated, spread morphology but did not affect microtubule networks. Rac2D57N also inhibited lipopolysaccharide-stimulated p38 kinase activation. Examination of guanine nucleotide binding to recombinant Rac2D57N revealed reduced dissociation of GDP and association of GTP. Coimmunoprecipitation studies of Rac2D57N with RhoGDI(alphap) and Tiam1 demonstrated increased binding of Rac2D57N to these upstream regulators of Rac signaling relative to the wild type. Enhanced binding of Rac2D57N to its upstream regulators would inhibit Rac-dependent effects on actin cytoskeletal dynamics and p38 kinase signaling.


http://jcs.biologists.org/cgi/content/abstract/118/4/743
Premature senescence of human diploid fibroblasts (HDFs) can be induced by exposures to a variety of oxidative stress and DNA damaging agents. In this study we developed a robust model of UVB-induced premature senescence of skin HDFs. After a series of 10 subcytotoxic (non-proapoptotic) exposures to UVB at 250 mJ/cm², the so-called biomarkers of senescence were markedly expressed: growth arrest, senescence-associated {beta}-galactosidase activity, senescence-associated gene overexpression, deletion in mitochondrial DNA. A set of 44 stress- and senescence-associated genes were found to be differentially expressed in this model, among which clusterin/apolipoprotein J (apo J) and transforming growth factor-{beta}1 (TGF-{beta}1). Transfection of apo J cDNA provided protection against premature senescence-inducing doses of UVB and other stressful agents. Neutralizing antibodies against TGF-{beta}1 or its receptor II (T{beta}RII) sharply attenuated the senescence-associated features, suggesting a role for TGF-{beta}1 in UVB-induced premature senescence. Both the latent and active forms of TGF-{beta}1 were increased with time after the last UVB stress. Proteasome inhibition was ruled out as a potential mechanism of UVB-induced stress-induced premature senescence (SIPS). This model represents an alternative in vitro model in photoaging research for screening potential anti-photoaging compounds.


http://jcs.biologists.org/cgi/content/abstract/117/20/4717

Gap junctions are connexin-formed channels that play an important role in intercellular communication in most cell types. In the immune system, specifically in macrophages, the expression of connexins and the establishment of functional gap junctions are still controversial issues. Macrophages express P2X7 receptors that, once activated by the binding of extracellular ATP, lead to the opening of transmembrane pores permeable to molecules of up to 900 Da. There is evidence suggesting an interplay between gap junctions and P2 receptors in different cell systems. Thus, we used ATP-sensitive and -insensitive J774.G8 macrophage cell lines to investigate this interplay. To study junctional communication in J774-macrophage-like cells, we assessed cell-to-cell communication by microinjecting Lucifer Yellow. Confluent cultures of ATP-sensitive J774 cells (ATP-s cells) are coupled, whereas ATP-insensitive J774 cells (ATP-i cells), derived by overexposing J774 cells to extracellular ATP until they do not display the phenomenon of ATP-induced permeabilization, are essentially uncoupled. Western-blot and reverse-transcription polymerase chain reaction assays revealed that ATP-s and ATP-i cells express connexin43 (Cx43), whereas only ATP-s cells express the P2X7 receptor. Accordingly, ATP-i cells did not display any detectable ATP-induced current under whole-cell patch-clamp recordings. Using immunofluorescence microscopy, Cx43 reactivity was found at the cell surface and in regions of cell-cell contact of ATP-s cells, whereas, in ATP-i cells, Cx43 immunoreactivity was only present in cytosolic compartments. Using confocal microscopy, it is shown here that, in ATP-s cells as well as in peritoneal macrophages, Cx43 and P2X7 receptors are co-localized to the membrane of ATP-s cells and peritoneal macrophages.


http://jcs.biologists.org/cgi/content/abstract/116/15/3165

In primary cultures of rat cerebellar granule cells with a functional network of glutamatergic neurons, the expression pattern of the different subunits of nitric-oxide (NO)-sensitive guanylyl cyclase changes during cell differentiation. These cells express the {alpha}1, {alpha}2 and {beta}1
subunits of NO-sensitive guanylyl cyclase and synthesize cyclic guanosine monophosphate (cGMP) in response to exogenous or endogenous nitric oxide. In this study, we determined the protein content of the {alpha}1 and {beta}1 subunits and quantified {alpha}1, {alpha}2 and {beta}1 mRNA by reverse transcription coupled to a polymerase chain reaction (RT-PCR). Expression of the {beta}1 subunit increased with the degree of cell differentiation, although most marked changes occurred at the {alpha} subunit level. In cells freshly isolated from rat pups on postnatal day 7 (P7) the most abundant {alpha} subunit was {alpha}1, while {alpha}2 appeared as the predominant subunit of this type in cultured cells. N-methyl-D-aspartate (NMDA) receptor stimulation in 7- or 14-day-cultured cells led to the upregulation of guanylyl cyclase subunit mRNAs; {alpha}2 mRNA levels undergoing most significant change. This enhanced subunit expression was accompanied by an increase in the amount of cGMP synthesized in response to NO. Thus, it seems that {alpha}2 subunits are increasingly expressed as granule cells mature. The presence of this subunit in the guanylyl cyclase heterodimer facilitates its localization at synaptic membranes, where the enzyme acts as a sensor for NO formed by the postsynaptic protein 95 (PSD-95)-associated neuronal NO synthase.


Integrin-linked kinase (ILK) is one of the signaling moieties that interact with the cytoplasmic domains of integrin {beta}1 and {beta}3 subunits. Integrin-mediated outside-in signals cooperate with vascular endothelial growth factor (VEGF) receptor to promote morphological changes, cell proliferation and motility in endothelial cells. In this report we demonstrate that VEGF-induced vessel morphogenesis of human umbilical vein endothelial cells (HUVEC) was inhibited by the transfection of a dominant negative, kinase-deficient ILK (ILK-KD), as well as by treatment with the phosphatidylinositol 3-kinase inhibitor LY294002. VEGF induced phosphorylation of protein kinase B (PKB/Akt), a regulator of cell survival and apoptosis, on serine 473, but not on threonine 308, in an ILK-dependent manner. Furthermore, transfection of antisense ILK (ILK-AS) blocked the survival effect of VEGF in annexin-V binding assays, and a VEGF-mediated decrease in caspase activity was reversed by both ILK-KD and ILK-AS as measured by a homogeneous caspase-3/7 assay. We also demonstrate that both chemotactic migration and cell proliferation of HUVEC induced by VEGF were suppressed by the inhibition of ILK. We conclude that ILK plays an important role in vascular morphogenesis mediated by VEGF.


Tumour angiogenesis is a complex process based upon a sequence of interactions between tumour cells and endothelial cells. To model tumour/endothelial-cell interactions, we co-cultured U87 human glioma cells with human umbilical vein endothelial cells (HUVECs). U87 cells induced an 'activated' phenotype in HUVECs, including an increase in proliferation, migration and net-like formation. Activation was observed in co-cultures where cells were in direct contact and physically separated, suggesting an important role for soluble factor(s) in the phenotypic and genotypic changes observed. Expression profiling of tumour-activated endothelial cells was evaluated using cDNA arrays and confirmed by quantitative PCR. Matching pairs of receptors/ligands were found to be coordinately expressed, including TGF{beta}RII with TGF{beta}3, FGFRII and cysteine-rich fibroblast growth factor receptor (CRF-1) with FGF7 and
FGF12, CCR1, CCR3, CCR5 with RANTES and calcitonin receptor-like gene (CALCRL) with adrenomedullin. Consistent with cDNA array data, immunohistochemical staining of expressed proteins revealed the upregulation of Tie-2 receptor in vitro and in vivo. Our data suggest that tumour-induced activation of quiescent endothelial cells involves the expression of angiogenesis-related receptors and the induction of autocrine growth loops. We suggest that tumour cells release growth factors that induce endothelial cells to express specific ligands and their cognate receptors coordinately.


http://jcs.biologists.org/cgi/content/abstract/115/17/3427

Macro- and microvascular endothelial cells (EC) formed tubular structures when cultured within a 3D fibrin matrix, a process that was enhanced by vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor/scatter factor (HGF/SF) and an angiogenic cocktail composed of nine angiogenic factors. Endothelial tubulogenesis was also increased in co-culture with tumour cells such as U87 glioma cells, but not with non-tumorigenic cell types such as Madin-Darby canine kidney (MDCK) epithelial cells. VEGF/FGF-2-stimulated tube formation was dependent on metalloproteinsc function [it is inhibited by the addition of tissue inhibitor of metalloproteinsases-2 (TIMP-2)], whereas aprotinin, E64 [trans-epoxysuccinyl-L-leucylamido (4-guanidino)-butane] and pepstatin had no effect. In addition, TIMP-4 also inhibited tubulogenesis, but TIMP-1 or the C-terminal haemopexin domain of matrix metalloproteinase-2 (MMP-2) (PEX) and an anti-MMP-2 function-blocking antibody were unable to block tube formation. This suggests that MMP-2 and other soluble MMPs are not essential for tubulogenesis in fibrin gels, instead TIMP-1-insensitive MMPs, such as members of the membrane type-MMPs (MT-MMP) sub-group (MT1-, MT2-, MT3- or MT5-MMP), are required for this process. Further support for a role for MT1-MMP in endothelial tubulogenesis is that recombinant Y36G N-terminal TIMP-2 mutant protein, which retains an essentially unaltered apparent inhibition constant (Kiapp) for several MMPs compared to wild-type N-TIMP-2 but is a 40-fold poorer inhibitor of MT1-MMP, was unable to block tubulogenesis. Furthermore, when EC were cultured within fibrin gels, the mRNA levels of several MMPs (including MT1-MMP, MT2-MMP, MT3-MMP and MMP-2) increased during tubulogenesis. Therefore MT-MMPs and specifically MT1-MMP are likely candidates for involvement during endothelial tubulogenesis within a fibrin matrix, and thus their blockade may be a viable strategy for inhibition of angiogenesis.


http://jcs.biologists.org/cgi/content/abstract/117/8/1567

The growth and survival of the preimplantation mammalian embryo may be regulated by several autocrine trophic factors that have redundant or overlapping actions. One of the earliest trophic factors to be produced is embryo-derived platelet-activating factor (1-O-alky-2-acetyl-sn-glyceryl-3-phosphocholine). The addition of platelet-activating factor to embryo culture media exerted a trophic effect, but structurally related lipids (3-O-alky-2-acetyl-sn-glyceryl-1-phosphocholine, 1-O-alky-sn-glyceryl-3-phosphocholine, octadecyl-phosphocholine) had no effect. Platelet-activating factor induced a pertussis toxin-sensitive [Ca2+]i transient in two-cell embryos that did not occur in platelet-activating factor-receptor null (Pafr-/-) genotype embryos. Fewer Pafr-/- mouse zygotes developed to the blastocyst stage in vitro compared with Pafr+/+ zygotes (P<0.02), those that
developed to blastocysts had fewer cells (P<0.001) and more cells with fragmented nuclei (P<0.001). The inhibition of 1-O-phosphatidylinositol 3-kinase (LY294002 (3 {micro}M and 15 {micro}M) and wortmannin (10 nM and 50 nM)) caused a dose-dependent inhibition of platelet-activating factor-induced [Ca2+]i transients (P<0.001). The two-cell embryo expressed 1-O-phosphatidylinositol 3-kinase catalytic subunits p110{alpha}, {beta}, {gamma} and {delta}, and regulatory subunits p85(alpha) and (beta). LY294002 and wortmannin each caused a significant reduction in the proportion of embryos developing to the morula and blastocyst stages in vitro, reduced the number of cells within each blastocyst, and significantly increased the proportion of cells in blastocysts with fragmented nuclei. The results indicate that embryo-derived platelet-activating factor (and other embryotrophic factors) act through its membrane receptor to enhance embryo survival through a 1-O-phosphatidylinositol 3-kinase-dependent survival pathway.

http://jcs.biologists.org/cgi/content/abstract/116/12/2421

It was previously reported that a midregion domain of parathyroid hormone-related protein (PTHrP), that is, [67-86]-amide, is able to restrain growth and promote matrigel penetration by the 8701-BC cell line, derived from a biopsy fragment of a primary ductal infiltrating carcinoma of the human breast, and that cell invasion in vitro is drastically impaired by inactivation of urokinase-plasminogen activator (uPa). In this study we started a more detailed investigation of the possible effects on gene expression arising from the interaction between PTHrP [67-86]-amide and 8701-BC breast cancer cells by a combination of conventional-, differential display-and semi-quantitative multiplex-polymerase chain reaction (PCR) assays. We present here the first evidence that the upregulation of some stress-related genes, most noticeably heat shock factor binding protein-1 (hsbp1) and heat shock protein 90 (hsp-90), is involved in the acquisition of an in vitro more invasive phenotype by cells treated with midregion PTHrP. This is conceivably accomplished by sequestering and inactivating heat shock factor-1 (hsf1) which is able to recognize Ets transcription-factor-binding sites present in some gene promoters, such as those of uPa and matrix metalloprotease-1 (MMP-1). In fact, our data show that incubation of PTHrP [67-86]-amide-treated cells with either antisense hsbp1-oligonucleotide or geldanamycin, an hsp90-inactivating antibiotic, results in downregulation of uPa and upregulation of MMP-1, and in a prominent inhibition of cell invasion in matrigel-containing Transwell chambers. Alternatively, incubation of untreated 8701-BC cells with quercetin, a flavonoid known to decrease the amount of free hsf1, is found to induce upregulation of uPa and downregulation of MMP-1, and an increase of matrigel invasion by cells, thus providing further supporting data of the involvement of hsf unavailability on the modulation of uPa and MMP-1 expression and on cell invasive behaviour. These studies confirm a previous postulate that over-secretion of uPa, rather than of other extracellular proteases, is a primary condition for the increase of invasive activity triggered by PTHrP [67-86]-amide in vitro, and support a role for midregion forms of PTHrP in potentially affecting pathological mammary growth and differentiation. They also identify two new key protagonists in the complex scenario of breast tumor cell invasiveness in vitro, that is, hsbp1 and hsp90, which deserve further and more extensive studies as potential and attractive molecular targets for anti-breast cancer treatments.

http://jcs.biologists.org/cgi/content/abstract/115/3/563
TgMIC6, TgMIC7, TgMIC8 and TgMIC9 are members of a novel family of transmembrane proteins localized in the micronemes of the protozoan parasite Toxoplasma gondii. These proteins contain multiple epidermal growth factor-like domains, a putative transmembrane spanning domain and a short cytoplasmic tail. Sorting signals to the micronemes are encoded in this short tail. We established previously that TgMIC6 serves as an escorter for two soluble adhesins, TgMIC1 and TgMIC4. Here, we present the characterization of TgMIC6 and three additional members of this family, TgMIC7, -8 and -9. Consistent with having sorting signals localized in its C-terminal tail, TgMIC6 exhibits a classical type I membrane topology during its transport along the secretory pathway and during storage in the micronemes. TgMIC6 is processed at the N-terminus, probably in the trans-Golgi network, and the cleavage site has been precisely mapped. Additionally, like other members of the thrombospondin-related anonymous protein family, TgMIC2, TgMIC6 and TgMIC8 are proteolytically cleaved near their C-terminal domain upon discharge by micronemes. We also provide evidence that TgMIC8 escorts another recently described soluble adhesin, TgMIC3. This suggests that the existence of microneme protein complexes is not an exception but rather the rule. TgMIC6 and TgMIC8 are expressed in the rapidly dividing tachyzoites, while TgMIC7 and TgMIC9 genes are predominantly expressed in bradyzoites, where they presumably also serve as escorters.


http://jcs.biologists.org/cgi/content/abstract/116/19/4021

To investigate the mechanism of chromatin assembly at human centromeres, we isolated cultured human cell lines in which a transfected alpha-satellite (alphoid) YAC was integrated ectopically into the terminal region of host chromosome 16, where it was stably maintained. Centromere activity of the alphoid YAC was suppressed at ectopic locations on the host chromosome, as indicated by the absent or reduced assembly of CENP-A and -C. However, long-term culture in selective medium, or short-term treatment with the histone deacetylase inhibitor Trichostatin A (TSA), promoted the re-assembly of CENPA, -B and -C at the YAC site and the release of minichromosomes containing the YAC integration site. Chromatin immunoprecipitation analyses of the re-formed minichromosome and the alphoid YAC-based stable human artificial chromosome both indicated that CENP-A and CENP-B assembled only on the inserted alphoid array but not on the YAC arms. On the YAC arms at the alphoid YAC integration sites, TSA treatment increased both the acetylation level of histone H3 and the transcriptional level of a marker gene. An increase in the level of transcription was also observed after long-term culture in selective medium. These activities, which are associated with changes in chromatin structure, might reverse the suppressed chromatin state of the YAC at ectopic loci, and thus might be involved in the epigenetic change of silent centromeres on ectopic loci.


http://jcs.biologists.org/cgi/content/abstract/115/17/3491

During testicular development, fetal and adult populations of Leydig cells arise sequentially. Previous studies have shown that androgen action is required for normal steroidogenic activity in the mouse testis. Therefore, to determine the role of androgens in regulating fetal and adult Leydig cell differentiation and function, Leydig development has been measured in mice lacking functional androgen receptors (AR-null). The Leydig cell number was normal on day 5 after birth in AR-null mice but failed to increase normally thereafter and was about 30% of the control level on day 20 and about 60% of control level in adult animals. Levels of 15 different mRNA species
expressed specifically in Leydig cells were measured by real-time PCR in AR-null and control animals. Expression levels of all mRNA species were normal on day 5 when only fetal Leydig cells are present. In older animals, which contain predominantly adult Leydig cells, five of the mRNA species (3(β)-hydroxysteroid dehydrogenase (3(β)HSD) type 1, cytochrome P450sc, renin, STAR protein and luteinising hormone receptor) were expressed at normal or increased levels in AR-null mice. All other mRNA species measured showed significantly reduced expression in older animals, and three of these mRNA species (17(β)-hydroxysteroid dehydrogenase type III, prostaglandin D (PGD)-synthetase and 3(β)HSD type VI), which are only expressed in the adult population of Leydig cells, were barely detectable in the adult AR-null mouse. The results show that in the absence of androgen receptors, fetal Leydig cell function is normal, but there is a developmental failure of adult Leydig cell maturation, with cells only acquiring partial characteristics of the adult population.


http://jcs.biologists.org/cgi/content/abstract/118/2/433

Neurite elongation and branching are key cellular events during brain development as they underlie the formation of a properly wired neuronal network. Here we report that the receptor tyrosine kinases Ror1 and Ror2 modulate the growth of neurites as well as their branching pattern in hippocampal neurons. Upon Ror1 or Ror2 suppression using antisense oligonucleotides or RNA interference (RNAi), neurons extended shorter and less branched minor processes when compared to those in control cells. In addition, Ror-depleted cells elongated longer, albeit less branched, axons than seen in control cells. Conversely, Ror overexpression both in non-neuronal cells and in hippocampal neurons resulted in the enhanced extension of short and highly branched processes. These phenotypes were accompanied by changes in the microtubule-associated proteins MAP1B and MAP2. Taken together, these results support a novel role for Ror receptors as modulators of neurite extension in central neurons.


http://jcs.biologists.org/cgi/content/abstract/115/17/3457

Latent TGF-(β)-binding proteins (LTBPs) were initially identified through their binding to the growth factor. Three of the four known LTBPs are able to associate covalently with the small latent forms of TGF-(β) and mediate their efficient secretion. LTBPs have subsequently been found to associate with the extracellular matrix. We report here the cDNA cloning and characterization of the human LTBP-3 protein, which is the smallest LTBP. The hLTBP-3 gene consists of 28 exons, including one alternatively spliced exon. The splice variant contains an additional epidermal-growth-factor-like repeat in the C-terminus. The gene is transcribed to produce a ~4.6 kb mRNA, which is expressed at high levels in human heart, skeletal muscle, prostate and ovaries and in certain osteosarcoma and fibroblastic cell lines. Antibodies were generated against recombinant fragment of hLTBP-3 and used to detect the protein and its secretion from cultured COS-7 and osteosarcoma cells. Immunoblotting analysis indicated that efficient secretion of overexpressed hLTBP-3 from COS-7 cells required co-expression of TGF-(β)1, which resulted in the secretion of high molecular weight complexes of ~240 kDa. hLTBP-3 protein was secreted from cultured osteosarcoma cells as high molecular weight complexes rather than in the free form. Similar complexes were recognized with antibodies specific to (β)1′LAP. These findings indicate that human LTBP-3 has an essential role in the secretion and targeting of TGF-(β)1.
Kinectin is an integral transmembrane protein on the endoplasmic reticulum, binding to kinesin, interacting with Rho GTPase and anchoring the translation elongation factor-1 complex. There has been debate on the specific role(s) of kinectin in different species and cell types. Here we identified 15 novel kinectin isoforms in the mouse nervous system, constituting a family of alternatively spliced carboxyl-terminal variants. Isoform expression is subject to cell type- and developmental stage-specific regulation. We raised specific antibodies to the kinectin variants to characterise their differential intracellular localisation and discovered that certain kinectin isoforms are found in axons where kinectin was previously believed to be absent. We also demonstrated in vivo by overexpression and RNA interference assay that kinectin is selectively involved in the transport of specific types of organelles. A 160 kDa kinectin species is mainly concentrated in the endoplasmic reticulum, anchored via its transmembrane domain and is essential for endoplasmic reticulum membrane extension. A 120 kDa kinectin species is specifically associated with mitochondria, and its interaction with kinesin was found to influence mitochondrial dynamics. These findings contribute to a more unified view of kinectin function. They suggest that different cellular processes use specific kinectin isoforms to mediate intracellular motility and targeting by transient interaction with different motor proteins or other binding partners.

The physiological functions of the beta-amyloid precursor protein (APP) may include nuclear signaling. To characterize the role of the APP adaptor proteins Fe65, Jip1b, X11{alpha} (MINT1) and the chromatin-associated protein Tip60, we analyzed their interactions by confocal microscopy and co-immunoprecipitations. AICD corresponding to S3-cleaved APP bound to Fe65 that transported it to nuclei and docked it to Tip60. These proteins formed AICD-Fe65-Tip60 (AFT) complexes that were concentrated in spherical nuclear spots. {gamma}-Secretase inhibitors prevented AFT-complex formation with AICD derived from full-length APP. The APP adaptor protein Jip1b also transported AICD to nuclei and docked it to Tip60, but AICD-Jip1b-Tip60 (AJT) complexes had different, speckle-like morphology. By contrast, X11{alpha} trapped AICD in the cytosol. Induced AICD expression identified the APP-effector genes APP, BACE, Tip60, GSK3{beta} and KAI1, but not the Notch-effector gene Hes1 as transcriptional targets. These data establish a role for APP in nuclear signaling, and they suggest that therapeutic strategies designed to modulate the cleavage of APP affect AICD-dependent signaling.
Hypertension is a clinical syndrome characterized by increased vascular tone. However, the molecular mechanisms underlying vascular dysfunction during acquired hypertension remain unresolved. Localized intracellular Ca2+ release events through ryanodine receptors (Ca2+ sparks) in the sarcoplasmic reticulum are tightly coupled to the activation of large-conductance, Ca2+-activated K+ (BK) channels to provide a hyperpolarizing influence that opposes vasoconstriction. In this study we tested the hypothesis that a reduction in Ca2+ spark-BK channel coupling underlies vascular smooth muscle dysfunction during acquired hypertension. We found that in hypertension, expression of the {beta}1 subunit was decreased relative to the pore-forming {alpha} subunit of the BK channel. Consequently, the BK channels were functionally uncoupled from Ca2+ sparks. Consistent with this, the contribution of BK channels to vascular tone was reduced during hypertension. We conclude that downregulation of the {beta}1 subunit of the BK channel contributes to vascular dysfunction in hypertension. These results support the novel concept that changes in BK channel subunit composition regulate arterial smooth muscle function.


The paired-like homeobox gene expressed in embryonic stem cells Hesx1/HESX1 encodes a developmental repressor and is expressed in early development in a region fated to form the forebrain, with subsequent localization to Rathke's pouch, the primordium of the anterior pituitary gland. Mutations within the gene have been associated with septo-optic dysplasia, a constellation of phenotypes including eye, forebrain, and pituitary abnormalities, or milder degrees of hypopituitarism. We identified a novel homozygous nonconservative missense mutation (I26T) in the critical Engrailed homology repressor domain (eh1) of HESX1, the first, to our knowledge, to be described in humans, in a girl with evolving combined pituitary hormone deficiency born to consanguineous parents. Neuroimaging revealed a thin pituitary stalk with anterior pituitary hypoplasia and an ectopic posterior pituitary, but no midline or optic nerve abnormalities. This I26T mutation did not affect the DNA-binding ability of HESX1 but led to an impaired ability to recruit the mammalian Groucho homolog/Transducin-like enhancer of split-1 (Gro/TLE1), a crucial corepressor for HESX1, thereby leading to partial loss of repression. Thus, the novel pituitary phenotype highlighted here appears to be a specific consequence of the inability of HESX1 to recruit Groucho-related corepressors, suggesting that other molecular mechanisms govern HESX1 function in the forebrain.


Acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) is one of two known DGAT enzymes that catalyze the final step in mammalian triglyceride synthesis. DGAT1-deficient mice are resistant to diet-induced obesity through a mechanism involving increased energy expenditure. Here we show that these mice have decreased levels of tissue triglycerides, as well as increased
sensitivity to insulin and to leptin. Importantly, DGAT1 deficiency protects against insulin resistance and obesity in agouti yellow mice, a model of severe leptin resistance. In contrast, DGAT1 deficiency did not affect energy and glucose metabolism in leptin-deficient (ob/ob) mice, possibly due in part to a compensatory upregulation of DGAT2 expression in the absence of leptin. Our results suggest that inhibition of DGAT1 may be useful in treating insulin resistance and leptin resistance in human obesity.


http://www.jci.org/cgi/content/abstract/109/2/175

Acyl CoA:diacylglycerol acyltransferase (DGAT) is a ubiquitously expressed enzyme that catalyzes the final reaction in the major pathways of triglyceride synthesis. Mice lacking DGAT1 (Dgat-/-) demonstrate significant changes in lipid metabolism in several tissues, including the skin. Here we report the effects of DGAT1 deficiency on fur and sebaceous glands. Adult Dgat-/- mice had dry fur and hair loss, which were associated with atrophic sebaceous glands and fur lipid abnormalities. As a result, Dgat-/- mice had impaired water repulsion and defective thermoregulation after water immersion. These phenotypes were mostly absent in Dgat-/- mice with leptin deficiency, indicating an unexpected role for leptin in modulating the skin phenotype. Our findings indicate that DGAT1 plays an important role in normal fur and sebaceous gland physiology and provide evidence that leptin modulates these processes in the skin.


http://www.jci.org/cgi/content/abstract/113/12/1711

Uracil-DNA glycosylase (UNG) is involved in base excision repair of aberrant uracil residues in nuclear and mitochondrial DNA. Ung knockout mice generated by gene targeting are viable, fertile, and phenotypically normal and have regular mutation rates. However, when exposed to a nitric oxide donor, Ung-/- fibroblasts show an increase in the uracil/cytosine ratio in the genome and augmented cell death. After combined oxygen-glucose deprivation, Ung-/- primary cortical neurons have increased vulnerability to cell death, which is associated with early mitochondrial dysfunction. In vivo, UNG expression and activity are low in brains of naive WT mice but increase significantly after reversible middle cerebral artery occlusion and reperfusion. Moreover, major increases in infarct size are observed in Ung-/- mice compared with littermate control mice. In conclusion, our results provide compelling evidence that UNG is of major importance for tissue repair after brain ischemia.


http://www.jci.org/cgi/content/abstract/115/4/1068

In papillary thyroid carcinomas (PTCs), rearrangements of the RET receptor (RET/PTC) and activating mutations in the BRAF or RAS oncogenes are mutually exclusive. Here we show that the 3 proteins function along a linear oncogenic signaling cascade in which RET/PTC induces
RAS-dependent BRAF activation and RAS- and BRAF-dependent ERK activation. Adoptive activation of the RET/PTC-RAS-BRAF axis induced cell proliferation and Matrigel invasion of thyroid follicular cells. Gene expression profiling revealed that the 3 oncogenes activate a common transcriptional program in thyroid cells that includes upregulation of the CXCL1 and CXCL10 chemokines, which in turn stimulate proliferation and invasion. Thus, motile and mitogenic properties are intrinsic to transformed thyroid cells and are governed by an epistatic oncogenic signaling cascade.


http://www.jci.org/cgi/content/abstract/110/3/403

Ionizing radiation (IR) and radical oxygen intermediates (ROIs) activate the early growth response-1 (Egr1) promoter through specific cis-acting sequences termed CARG elements. Ad.Egr.TNF.11D, a replication-deficient adenoviral vector containing CARG elements cloned upstream of the cDNA for human recombinant TNF-(alpha) was used to treat human esophageal adenocarcinoma and rat colon adenocarcinoma cells in culture and as xenografts in athymic nude mice. Cisplatin, a commonly used chemotherapeutic agent, causes tumor cell death by producing DNA damage and generating ROIs. The present studies demonstrate induction of TNF-(alpha) production in tumor cells and xenografts treated with the combination of Ad.Egr.TNF.11D and cisplatin. The results show that the Egr1 promoter is induced by cisplatin and that this induction is mediated in part through the CARG elements. These studies also demonstrate an enhanced antitumor response without an increase in toxicity following treatment with Ad.Egr.TNF.11D and cisplatin, compared with either agent alone. Chemo-inducible cancer gene therapy thus provides a means to control transgene expression while enhancing the effectiveness of commonly used chemotherapeutic agents.


http://www.jci.org/cgi/content/abstract/114/10/1467

Hailey-Hailey disease (HHD) is an autosomal dominant trait characterized by erythematous and oozing skin lesions preponderantly involving the body folds. In the present unusual case, however, unilateral segmental areas along the lines of Blaschko showing a rather severe involvement were superimposed on the ordinary symmetrical phenotype. Based on this observation and similar forms of mosaicism as reported in other autosomal dominant skin disorders, we postulated that in such cases, 2 different types of segmental involvement can be distinguished. Accordingly, the linear lesions as noted in the present case would exemplify type 2 segmental HHD. In the heterozygous embryo, loss of heterozygosity occurring at an early developmental stage would have given rise to pronounced linear lesions reflecting homozygosity or hemizygosity for the mutation. By analyzing DNA and RNA derived from blood and skin samples as well as keratinocytes of the index patient with various molecular techniques including RT-PCR, real-time PCR, and microsatellite analysis, we found a consistent loss of the paternal wild-type allele in more severely affected segmental skin regions, confirming this hypothesis for the first time, to our knowledge, at the molecular and cellular level.
The cardiac pacemaker current If is a major determinant of diastolic depolarization in sinus nodal cells and has a key role in heartbeat generation. Therefore, we hypothesized that some forms of "idiopathic" sinus node dysfunction (SND) are related to inherited dysfunctions of cardiac pacemaker ion channels. In a candidate gene approach, a heterozygous 1-bp deletion (1631delC) in exon 5 of the human HCN4 gene was detected in a patient with idiopathic SND. The mutant HCN4 protein (HCN4-573X) had a truncated C-terminus and lacked the cyclic nucleotide-binding domain. COS-7 cells transiently transfected with HCN4-573X cDNA indicated normal intracellular trafficking and membrane integration of HCN4-573X subunits. Patch-clamp experiments showed that HCN4-573X channels mediated If-like currents that were insensitive to increased cellular cAMP levels. Coexpression experiments showed a dominant-negative effect of HCN4-573X subunits on wild-type subunits. These data indicate that the cardiac If channels are functionally expressed but with altered biophysical properties. Taken together, the clinical, genetic, and in vitro data provide a likely explanation for the patient's sinus bradycardia and the chronotropic incompetence.

The mitochondrial genome encodes 13 essential subunits of the respiratory chain and has remarkable genetics based on uniparental inheritance. Within human populations, the mitochondrial genome has a high rate of sequence divergence with multiple polymorphic variants and thus has played a major role in examining the evolutionary history of our species. In recent years it has also become apparent that pathogenic mitochondrial DNA (mtDNA) mutations play an important role in neurological and other diseases. Patients harbor many different mtDNA mutations, some of which are mtDNA mutations, some of which are inherited, but others that seem to be sporadic. It has also been suggested that mtDNA mutations play a role in aging and cancer, but the evidence for a causative role in these conditions is less clear. The accumulated data would suggest, however, that mtDNA mutations occur on a frequent basis. In this article we describe a new phenomenon: the accumulation of mtDNA mutations in human colonic crypt stem cells that result in a significant biochemical defect in their progeny. These studies have important consequences not only for understanding of the finding of mtDNA mutations in aging tissues and tumors, but also for determining the frequency of mtDNA mutations within a cell.
receptor (α1A-AR) and show its functional coupling to plasmalemmal cationic channels via direct diacylglycerol (DAG) gating. In both cell types, agonist-mediated stimulation of α1A-ARs and DAG analogues activated similar cationic membrane currents and Ca2+ influx. These currents were sensitive to the α1A-AR antagonists, prazosin and WB4101, and to transient receptor potential (TRP) channel blockers, 2-aminophenyl borate and SK&F 96365. Chronic activation of α1A-ARs enhanced LNCaP cell proliferation, which could be antagonized by α1A-AR and TRP inhibitors. Collectively, our results suggest that α1-ARs play a role in promoting hPCE cell proliferation via TRP channels.

http://www.jci.org/cgi/content/abstract/112/4/517

Stepwise degradation of the invariant chain (li) is required for the binding of antigenic peptides to MHC class II molecules. Cathepsin (Cat) L in the murine thymus and Cat S in peripheral APCs have both been implicated in the last step of li degradation that gives rise to the class II-associated invariant chain peptides (CLIP). Cat V has been recently described as highly homologous to Cat L and exclusively expressed in human thymus and testis, but with no mouse orthologue. We report that Cat V is the dominant cysteine protease in cortical human thymic epithelial cells, while Cat L and Cat S seem to be restricted to dendritic and macrophage-like cells. Active Cat V in thymic lysosomal preparations was demonstrated by active-site labeling. Recombinant Cat V was capable of converting li into CLIP efficiently, suggesting that Cat V is the protease that controls the generation of αβ-CLIP complexes in the human thymus, in analogy to Cat L in mouse. Comparison of Cat V expression between thymi from patients with myasthenia gravis and healthy controls revealed a significantly higher expression level in the pathological samples, suggesting a potential involvement of this protease in the immunopathogenesis of myasthenia gravis, an autoimmune disease almost invariably associated with thymic pathology.

http://www.jci.org/cgi/content/abstract/109/7/905

Chemokines are involved in recruitment and activation of hematopoietic cells in sites of infection and inflammation. The M3 gene of the γ-herpesvirus γHV68 encodes an abundant secreted protein that binds CC chemokines with high affinity. We report here that this gene is essential for efficient induction of lethal meningitis by γHV68. An M3 mutant γHV68 (γγHV68-M3.stop) was 100-fold less virulent than wild-type or marker rescue control ((γγHV68-M3.MR) viruses after intracerebral inoculation. After intracerebral inoculation, γHV68-M3.MR grew to lower titers than γHV68 or γHV68-M3.MR in the brain but spread to and grew normally in the spleen and lung. Expression of several CC chemokines was significantly induced in the CNS by γHV68 infection. Consistent with M3 acting by blockade of CC chemokine action, γHV68 induced a neutrophilic meningeal inflammatory infiltrate, while γHV68-M3.stop induced an infiltrate in which lymphocytes and macrophages predominated. In contrast to the important role of M3 in lethal meningitis, M3 was not required for establishment or reactivation from latent infection or induction of chronic arteritis. These data suggest a role for chemokines in the protection of the nervous system from viral infection and that the M3 protein acts in a tissue-specific fashion during acute but not chronic γHV68 infection to limit CC chemokine-induced inflammatory responses.
Mutations in MEF2A have been implicated in an autosomal dominant form of coronary artery disease (adCAD1). In this study we sought to determine whether severe mutations in MEF2A might also explain sporadic cases of coronary artery disease (CAD). To do this, we resequenced the coding sequence and splice sites of MEF2A in approximately 300 patients with premature CAD and failed to find causative mutations in the CAD cohort. However, we did identify the 21-bp MEF2A coding sequence deletion originally implicated in adCAD1 in 1 of 300 elderly control subjects without CAD. Further screening of approximately 1,500 additional individuals without CAD revealed 2 more subjects with the MEF2A 21-bp deletion. Genotyping of 19 family members of the 3 probands with the 21-bp deletion in MEF2A revealed that the mutation did not cosegregate with early CAD. These studies support that MEF2A mutations are not a common cause of CAD in white people and argue strongly against a role for the MEF2A 21-bp deletion in autosomal dominant CAD.

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PURPOSE: Increases in neu/erbB-2 have been implicated in breast cancer prognosis, but do not predict all recurrences. On the basis of evidence that p53 mutation is involved in the development of human neoplasia, we examined the prognostic value of p53 alterations in combination with neu/erbB-2 amplification. PATIENTS AND METHODS: A consecutive series of women were observed for recurrence and death (median follow-up of 85 months) and tumors from 543 individuals were analyzed for p53 mutation status and neu/erbB-2 amplification. Exons 4 through 10 of the p53 gene were analyzed by single-stranded conformational polymorphism and mutations were confirmed by DNA sequencing. The association of p53 mutation status and neu/erbB-2 amplification with risk of recurrence and death was examined in survival analyses with traditional and histologic markers as prognostic factors. RESULTS: p53 mutations occurred in 24.5% of the axillary node-negative breast carcinomas. Mutations were more frequent in carcinomas with neu/erbB-2 amplification: 38.9% compared with only 20.9% in those without neu/erbB-2 amplification. We found elevated risks of disease recurrence and overall mortality in patients with both p53 mutation and neu/erbB-2 amplification in their tumor compared with patients with neither or only one of the alterations. This increase persisted with adjustment for other prognostic factors (relative risk, 2.32; P =.002 for recurrence; relative risk, 2.22; P =.004 for death). CONCLUSION: Evaluation of tumors for p53 mutations may be beneficial to identify women at higher risk of disease recurrence and death when the tumor has neu/erbB-2 amplification, but in the absence of neu/erbB-2 amplification, the presence of p53 mutation may not provide additional independent prognostic information.

http://www.jco.org/cgi/content/abstract/21/7/1320

Purpose: To evaluate in vivo whether the expression of the human telomerase reverse transcriptase (hTERT) gene, the catalytic subunit of the telomerase complex, is predictive of response to chemotherapy in ovarian cancer patients. Patients and Methods: Fifty-nine advanced-stage ovarian cancer patients who were treated with platinum-based chemotherapy were studied. hTERT levels were evaluated by real-time reverse transcriptase polymerase chain reaction (RT-PCR) on tumor specimens obtained before the treatment. Variables were analyzed by the {chi}2 and Fisher's exact tests. Logistic regression analysis was also performed to account for the effects of all the covariates investigated (residual disease, stage, histotype, and grade). Results: Twenty-eight (47%) of the 59 tumors showed low hTERT levels, whereas 31 (53%) tumors displayed high hTERT levels. Seventy-five percent of complete responders showed high levels of hTERT expression, whereas 66% of partial responders or nonresponders exhibited low hTERT levels (P =.002). Only residual disease and hTERT expression were independent predictors of response (odds ratios, 13.455 and 7.586, respectively). The combination of these two parameters provides powerful predictive information: 18 of the 20 patients with residual disease more than 2 cm and low hTERT levels were partial responders or nonresponders, whereas 11 of the 12 patients with residual disease less than 2 cm and high hTERT levels showed a complete response ((chi)2 = 21,416; P <.00001). Conclusion: Our data indicate that hTERT expression, measured by real-time RT-PCR, is a possible independent marker of response to platinum-based therapy in advanced stage ovarian cancer patients. Prospective validation of this marker will be required to further define its predictive value.


http://www.jco.org/cgi/content/abstract/22/3/474

PURPOSE: The identification of malignant cells in effusions by conventional cytology is hampered by its limited sensitivity. The aim of this study was to improve tumor cell detection in effusions by molecular approaches. MATERIALS AND METHODS: A total of 157 effusions from patients with tumors and 72 effusions from patients without a history or evidence of malignancy were included in this study. All effusion specimens were evaluated in parallel by cytology, fluorescence in situ hybridization (FISH) for aneuploidy, and reverse-transcriptase polymerase chain reaction (RT-PCR) for expression of human mammaglobin (hMAM) and mammaglobin B (hMAM-B). RESULTS: In effusions from patients with tumors, the sensitivities of tumor cell detection by cytology, FISH, and hMAM and hMAM-B detection were 46.2%, 53.3%, 36.4%, and 57.7%, respectively. The corresponding specificities were 94.4%, 97.0%, 87.1%, and 88.6%. Notably, a high percentage of effusions containing malignant cells were in fact transudates, indicating the necessity for molecular diagnostic work-up of transudates collected from patients with tumors. Dependent on the tumor type, the use of appropriate marker combinations improved tumor cell detection in effusions significantly. By combining all four diagnostic tests, a positive test result indicating the presence of malignancy was achieved in 81.1%, with a fairly good specificity of 70.1%. CONCLUSION: Molecular techniques are definitely useful to detect malignancy in cytologically negative effusions. Tumor cell detection in effusions can be significantly improved by FISH and PCR techniques applying appropriate molecular markers. This finding should help to improve tumor staging, prognostic assessment, and treatment monitoring.

http://www.jco.org/cgi/content/abstract/23/6/1078

PURPOSE: We determined the maximum-tolerated dose (MTD) and the dose-limiting toxicities (DLT) of 17-allylamino-17-demethoxygeldanamycin (17-AAG) when infused on days 1, 8, and 15 of a 28-day cycle in advanced solid tumor patients. We also characterized the pharmacokinetics of 17-AAG, its effect on chaperone and client proteins, and whether cytochrome P450 (CYP) 3A5 and NAD(P)H:quinone oxidoreductase 1 (NQO1) polymorphisms affected 17-AAG disposition or toxicity. PATIENTS AND METHODS: An accelerated titration design was used. Biomarkers were measured in peripheral-blood mononuclear cells (PBMCs) at baseline and on days 1 and 15, and pharmacokinetic analysis was performed on day 1 of cycle 1. CYP3A5*3 and NQO1*2 genotypes were determined and correlated with pharmacokinetics and toxicity. RESULTS: Twenty-one patients received 52 courses at 11 dose levels. DLTs at 431 mg/m2 were grade 3 bilirubin (n = 1), AST (n = 1), anemia (n = 1), nausea (n = 1), vomiting (n = 1), and myalgias (n = 1). No tumor responses were seen. 17-AAG consistently increased heat shock protein (Hsp) 70 levels in PBMCs. At the MTD, the clearance and half-life (t1/2) of 17-AAG were 11.6 L/h/m2 and 4.15 hours, respectively; whereas the active metabolite 17-aminogeldanamycin had a t1/2 of 7.63 hours. The CYP3A5*3 and NQO1*2 polymorphisms were not associated with 17-AAG toxicity. The CYP3A5*3 polymorphism was associated with higher 17-AAG clearance. CONCLUSION: The MTD of weekly 17-AAG is 308 mg/m2. 17-AAG induced Hsp70 in PBMCs, indicating that Hsp90 has been affected. Further evaluation of 17-AAG is ongoing using a twice-weekly regimen, and this schedule of 17-AAG is being tested in combination with chemotherapy.


http://www.jco.org/cgi/content/abstract/21/1/106

Purpose: To establish the clinical significance of calcium binding proteins S100A2 and S100A4 during progression of human prostate adenocarcinoma. Patients and Methods: Expression pattern of S100A2 and S100A4 was determined in normal human prostate epithelial cells (NHPE); virally transformed prostate epithelial cells (PZ-HPV-7); several human prostate carcinoma cells (22Rv1, DU145, LNCaP, and PC3); tissue samples obtained during transurethral prostatic resection from patients with benign prostate hyperplasia (BPH), prostatitis, and adenocarcinoma; and paraffin-embedded sections from pair-matched benign and cancer specimens of different tumor grade. Results: High constitutive protein expression of S100A2 was observed in NHPE and PZ-HPV-7 cells, whereas its complete absence was observed in 22Rv1, DU145, LNCaP, and PC3 cells. Tissue samples of BPH and prostatitis exhibited higher mRNA and protein levels of S100A2 than low-grade cancer (Gleason score [&lt;=] 6), whereas a complete loss was observed in high-grade cancer specimens (Gleason score &gt; 6). Immunohistochemical analysis further confirmed high levels of S100A2 in benign tissues and a progressive loss with increasing tumor grade. The protein level of S100A4 was significantly higher in all carcinoma cells compared with NHPE and PZ-HPV-7 cells. The mRNA and protein level of S100A4 was significantly higher in high-grade cancer specimens compared with BPH, prostatitis, and low-grade cancer. The high levels of S100A4 observed in cancer tissue correlated with increasing tumor grade. Conclusion: Loss of S100A2 and increased expression of S100A4 may be an important event during progression of prostate cancer in humans.
PURPOSE: To evaluate the efficacy of rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) induction therapy in patients with newly diagnosed mantle-cell lymphoma (MCL). PATIENTS AND METHODS: From March 1997 to May 1999, 40 previously untreated patients with stage II through IV MCL were treated with six cycles of rituximab and CHOP chemotherapy in a phase II trial. Pretreatment and interval peripheral-blood (PB) and bone marrow (BM) specimens were also analyzed by polymerase chain reaction (PCR) for tumor-specific BCL-1/immunoglobulin H (IgH) translocations and clonal IgH rearrangements. Study end points included clinical and molecular response rates and long-term progression-free survival (PFS). RESULTS: Forty-eight percent of patients achieved a complete response (CR)/CR unconfirmed (CRu), and 48% of patients obtained a partial response (PR). However, 28 of the 40 patients have already relapsed or developed progressive disease with a median PFS of 16.6 months. Twenty-five patients had PCR-detectable BCL-1/IgH or clonal IgH products in PB or BM at diagnosis. Nine of the 25 informative patients had no evidence of PCR-detectable disease in PB or BM after rituximab and CHOP therapy. However, patients who achieved molecular remissions in PB or BM had PFS similar to patients without molecular remissions (16.5 v 18.8 months, P =.51). CONCLUSION: Favorable clinical and molecular response rates associated with rituximab and CHOP chemotherapy do not translate into prolonged PFS in MCL. Nevertheless, rituximab and combination chemotherapy may transiently clear PB or BM of detectable tumor cells, prompting additional consideration of antibody-based in vivo purging in subsequent clinical trials.


PURPOSE: To determine whether the application of two courses of cisplatin simultaneously with hyperfractionated radiotherapy improves the outcome in locally advanced and/or node-positive nonmetastatic carcinomas of the head and neck, compared with hyperfractionated radiotherapy alone. PATIENTS AND METHODS: From July 1994 to July 2000, 224 patients with squamous cell carcinomas of the head and neck (excluding nasopharynx and paranasal sinus) were randomly assigned to hyperfractionated radiotherapy (median dose, 74.4 Gy; 1.2 Gy twice daily) or the same radiotherapy combined with two cycles of concomitant cisplatin (20 mg/m2 on 5 days of weeks 1 and 5). The primary end point was time to any treatment failure; secondary end points were locoregional failure, metastatic relapse, overall survival, and late toxicity. RESULTS: There was no difference in radiotherapy between both treatment arms (74.4 Gy in 44 days). The full cisplatin dose was applied in 93% and 71% of patients during the first and second treatment cycles, respectively. Acute toxicity was similar in both arms. Median time to any treatment failure was not significantly different between treatment arms (19 months for combined treatment and 16 months for radiotherapy only, respectively) and the failure-free rate at 2.5 years was 45% and 33%, respectively. Locoregional control and distant disease-free survival were significantly improved with cisplatin (log-rank test, P =.039 and .011, respectively). The difference in overall survival did not reach significance (log-rank test, P =.147). Late toxicity was comparable in both treatment groups. CONCLUSION: The therapeutic index of hyperfractionated radiotherapy is improved by concomitant cisplatin.

http://www.jco.org/cgi/content/abstract/21/16/3084

Purpose: Despite its clinical success, methotrexate (MTX) therapy is associated with toxicities such as seizures, the pathogenesis of which remains unclear. It has been suggested that hyperhomocysteinemia is caused by MTX and is responsible for its neurotoxic effects. The purposes of this study were to explore whether hyperhomocysteinemia was related to MTX administration and toxicity and whether homocysteine or MTX toxicity differed by methylenetetrahydrofolate reductase (MTHFR) or reduced folate carrier (RFC) genetic polymorphisms. Patients and Methods: We studied 53 children with newly diagnosed acute lymphoblastic leukemia who were consecutively treated on a single clinical protocol that included two courses of high-dose MTX (high-dose methotrexate [HDMTX]; 2.5 or 5.0 g/m2 per day) as consolidation therapy. Results: The study participants' median plasma homocysteine concentrations at 23 and 44 hours after HDMTX (9.00 {micro}mol/L and 10.12 {micro}mol/L, respectively) were greater than the concentrations immediately before HDMTX (5.77 {micro}mol/L, P <.0001 for both comparisons). Seven days after HDMTX treatment, their plasma concentration returned to baseline. Nine patients experienced seizures, and five patients experienced thrombosis during the first 15 months of therapy, with a tendency for there to be higher plasma homocysteine in patients with seizures across all time points (P =.063) but not in patients with thrombosis (P =.59). We observed no significant differences in plasma or cerebrospinal fluid homocysteine levels or in toxicity based on the MTHFR 677C/T or RFC 80G/A genotypes. Conclusion: We conclude that homocysteine was transiently elevated after HDMTX and may be related to seizure risk in children with leukemia.


http://www.jco.org/cgi/content/abstract/23/10/2318

PURPOSE: The significance of low microsatellite instability (MSI-L) in colorectal cancer is poorly understood. No clear biologic distinction has been found between MSI-L and microsatellite stable (MSS) colorectal cancer, and these two phenotypes are usually combined when analyzed against the well-defined high MSI (MSI-H) phenotype. Evidence is emerging that an O6-methylguanine DNA methyltransferase (MGMT) gene defect is associated with MSI-L. Therefore, to further define this phenotype, we undertook a detailed analysis of the prognostic significance of MSI-L and loss of MGMT expression in colon cancer. PATIENTS AND METHODS: The study cohort was 183 patients with clinicopathologic stage C colon cancer who had not received adjuvant therapy. We analyzed MSI status, MGMT, and mismatch repair protein expression, as well as MGMT and p16 promoter hypermethylation. RESULTS: We showed that MSI-L defines a group of patients with poorer survival (P =.026) than MSS patients, and that MSI-L was an independent prognostic indicator (P =.005) in stage C colon cancer. Loss of MGMT protein expression was associated with the MSI-L phenotype but was not a prognostic factor for overall survival in colon cancer. p16 methylation was significantly less frequent in MSI-L than in MSI-H and MSS tumors and was not associated with survival. CONCLUSION: MSI-L characterizes a distinct subgroup of stage C colon cancer patients, including the MSI-L subset of proximal colon cancer, who have a poorer outcome. Neither the MGMT defect nor p16 methylation are likely to contribute to the worse prognosis of the MSI-L phenotype.

PURPOSE: To study safety, feasibility, and biologic activity of adenovirus-mediated p53 gene transfer in patients with bladder cancer. PATIENTS AND METHODS: Twelve patients with histologically confirmed bladder cancer scheduled for cystectomy were treated on day 1 with a single intratumoral injection of SCH 58500 (rAd/p53) at cystoscopy at one dose level (7.5 x 10^11 particles) or a single intravesical instillation of SCH 58500 with a transduction-enhancing agent (Big CHAP) at three dose levels (7.5 x 10^11 to 7.5 x 10^13 particles). Cystectomies were performed in 11 patients on day 3, and transgene expression, vector distribution, and biologic markers of transgene activity were assessed by molecular and immunohistochemical methods in tumors and normal bladder samples. RESULTS: Specific transgene expression was detected in tissues from seven of eight assessable patients treated with intravesical instillation of SCH 58500 but in none of three assessable patients treated with intratumoral injection of SCH 58500. Induction of RNA and protein expression of the p53 target gene p21/WAF1 was demonstrated in samples from patients treated with SCH 58500 instillation at higher dose levels. Distribution studies after intravesical instillation of SCH 58500 revealed both high transduction efficacy and vector penetration throughout the whole urothelium and into submucosal tumor cells. No dose-limiting toxicity was observed, and side effects were local and of transient nature. CONCLUSION: Intravesical instillation of SCH 58500 combined with a transduction-enhancing agent is safe, feasible, and biologically active in patients with bladder cancer. Studies to evaluate the clinical efficacy of this treatment in patients with localized high-risk bladder cancer are warranted.
PURPOSE: Inconsistent conclusions have been drawn about the clinical significance of micrometastases in lymph nodes (LN) of node-negative colorectal cancer (CRC) patients. We performed a comparative study of detection of micrometastases using immunohistochemistry (IHC) by anti-cytokeratin antibody and carcinoembryonic antigen (CEA)-specific reverse-transcriptase polymerase chain reaction (RT-PCR) in the same patients, in an attempt to move closer to their clinical application. PATIENTS AND METHODS: Sixty-four CRC patients, with RNA of good quality available from paraffin-embedded LN specimens, were selected from 84 stage II patients who underwent curative surgery between 1988 and 1996. We investigated associations between the presence of micrometastases by each method and prognosis. RESULTS: Micrometastases were detected in 19 (29.6%) of 64 patients by RT-PCR and in 35 (54.7%) of 64 patients by IHC. By RT-PCR analysis, patients exhibiting a positive band for CEA mRNA had a significantly worse prognosis than those who were RT-PCR-negative, with respect to both disease-free and overall survival (P = .027 and .015, respectively). By IHC analysis, the presence of micrometastasis did not predict patient outcome in terms of either disease-free or overall survival. Infiltrating pattern of tumor growth characteristic was significantly associated with shorter disease-free survival among various clinical or pathologic factors. By multivariate Cox regression analysis, micrometastasis detected by RT-PCR and the Crohn's-like lymphoid reaction were both independent prognostic factors. CONCLUSION: Micrometastases detected by RT-PCR, but not IHC, may be of clinical value in identifying patients who may be at high risk for recurrence of CRC and who are therefore likely to benefit from systemic adjuvant therapy.


PURPOSE: Overweight (body mass index [BMI] 25 to 29 kg/m2) and obesity (BMI [≥] 30 kg/m2) frequently follow treatment for childhood acute lymphoblastic leukemia (ALL). Recent studies suggest that risk is most apparent in females treated with cranial radiation at a younger age. Because radiation at a young age may affect the hypothalamus causing leptin receptor insensitivity, we hypothesized that a polymorphism in the leptin receptor (LEPR) gene, Gln223Arg, might influence susceptibility to obesity in survivors of childhood ALL. PATIENTS AND METHODS: We genotyped 600 non-Hispanic white adult ALL survivors enrolled onto the Childhood Cancer Survivor Study. BMI was compared between those with two copies of the Arg allele to those who had at least one copy of the Gln allele. RESULTS: Female survivors with BMI ≥ 25 kg/m2 were more likely Arg homozygous than those with BMI less than 25 kg/m2 (24% vs 12%; P = .007). This difference was not observed in males. Moreover, among females treated with ≥ 20 Gy cranial radiation, Arg/Arg individuals had six times higher odds of having BMI ≥ 25 kg/m2 (95% CI, 2.1 to 22.0) than those with a Gln allele (P = .04 for interaction). CONCLUSION: LEPR polymorphism may influence obesity in female survivors of childhood ALL, particularly those exposed to cranial radiation. Because obesity is associated with increased morbidity and mortality in later life, identification of children at high risk might allow for early targeted interventions.

Purpose: To study whether hematopoietic stem-cell transplantation (HSCT) after reduced-intensity conditioning is effective and tolerable in patients with advanced chronic lymphocytic leukemia (CLL). Patients and Methods: Thirty patients with advanced B-cell CLL were included into the study. After reduced-intensity conditioning with fludarabine, busulfan, and antithymocyte globulin, patients received a transplant from related (n = 15) or unrelated donors (n = 15). Minimal residual disease (MRD) was monitored with a clone-specific polymerase chain reaction. Results: After a median follow-up of 2 years, 23 patients are alive (to date). Neutrophil and platelet engraftment occurred after a median of 17.5 and 15 days, respectively. Acute graft-versus-host disease (GVHD) grade 2 to 4 was observed in 17 patients (56%), and chronic GVHD was observed in 21 patients (75%). Twelve patients (40%) achieved a complete remission (CR), and 16 patients (53%) achieved a partial remission. Late CR occurred up to 2 years after transplantation. MRD was monitored in eight patients with CR. All patients achieved a molecular CR. At last follow-up, six patients were in ongoing molecular CR. Causes of death were treatment-related complications in four patients and progressive disease in three patients. The probability of overall survival, progression-free survival, and nonrelapse mortality at 2 years was 72% (95% confidence interval [CI], 54% to 90%), 67% (95% CI, 49% to 85%), and 15% (95% CI, 1% to 29%), respectively. Conclusion: Treatment-related mortality after reduced-intensity conditioning followed by allogeneic HSCT was low. The procedure induced molecular remissions in patients with advanced CLL. The observation of late remissions provided evidence of a graft-versus-leukemia effect.


PURPOSE: Mutated KIT and platelet-derived growth factor receptor alpha (PDGFR(alpha)) tyrosine kinases are the principal targets for imatinib mesylate in the treatment of gastrointestinal stromal tumors (GISTs). The frequency of activating KIT and PDGFRA gene mutations in most other histologic types of human cancer is not known. MATERIALS AND METHODS: KIT exons 9, 11, 13, and 17 and PDGFRA exons 11 and 17 of 334 human cancers were screened for mutations using sensitive denaturing high-performance liquid chromatography (DHPLC). In addition, all KIT exons from 9 to 21 of 115 tumors were screened. Thirty-two histologic tumor types were examined. Samples with abnormal findings in DHPLC were sequenced. Immunostaining for the KIT protein (CD117) was performed in 322 (96.4%) of the 334 cases. RESULTS: Of the 3,039 exons screened, only 17 had mutation. All 17 cases with either mutated KIT (n = 15) or PDGFRA (n = 2) were histologically GIST tumors, whereas none of the other histologic types of cancer (n = 316) harbored KIT or PDGFRA mutation. KIT immunostaining was rarely positive except in GISTs (18 of 18), small-cell lung cancer (10 of 30; 33%), and testicular teratocarcinoma (four of 17; 24%). Wild-type KIT gene amplification or chromosome 4 aneuploidy was common (seven of 12) in non-GIST tumors with strong KIT protein expression when studied with fluorescence in situ hybridization. CONCLUSION: Despite frequent KIT protein expression in some tumor types, KIT and PDGFRA gene mutations are uncommon in most human cancers. Cancer KIT expression is frequently associated with multiple copies of the wild-type KIT gene.

Purpose: Analysis of circulating DNA in plasma can provide a useful marker for earlier lung cancer detection. This study was designed to assess the sensitivity and specificity of a quantitative molecular assay of circulating DNA to identify patients with lung cancer and monitor their disease. Materials and Methods: The amount of plasma DNA was determined through the use of real-time quantitative polymerase chain reaction (PCR) amplification of the human telomerase reverse transcriptase gene (hTERT) in 100 non-small-cell lung cancer patients and 100 age-, sex-, and smoking-matched controls. Screening performance of the assay was calculated through the receiver operating characteristic (ROC) curve. Odds ratios were calculated using conditional logistic regression analysis. Results: Median concentration of circulating plasma DNA in patients was almost eight times the value detected in controls (24.3 v 3.1 ng/mL). The area under the ROC curve was 0.94 (95% CI, 0.907 to 0.973). Plasma DNA was a strong risk factor for lung cancer; concentrations in the upper tertile were associated with an 85-fold higher risk than were those in the lowest tertile. Conclusion: This study shows that higher levels of free circulating DNA can be detected in patients with lung cancer compared with disease-free heavy smokers by a PCR assay, and suggests a new, noninvasive approach for early detection of lung cancer. Levels of plasma DNA could also identify higher-risk individuals for lung cancer screening and chemoprevention trials.


http://www.jco.org/cgi/content/abstract/22/23/4717

PURPOSE: The unique immunoglobulin idiotype (Id) expressed by each B-cell lymphoma is a target for immunotherapy. Vaccination with Id induces humoral and/or cellular anti-Id immune responses. However, the clinical impact of these anti-Id immune responses is unknown. We and others have previously reported that immunoglobulin G Fc receptor (Fc(gamma)R) polymorphisms predict the clinical response of lymphoma patients to passive anti-CD20 antibody infusions. In this study, we tested whether anti-Id immune responses or Fc(gamma)R polymorphisms associate with clinical outcome of patients who received Id vaccination.

PATIENTS AND METHODS: We analyzed 136 patients with follicular lymphoma who had received Id vaccination. The anti-Id immune responses were measured and Fc(gamma)RIIIa and Fc(gamma)RIIa polymorphisms were determined and correlated with clinical outcome for these patients. RESULTS: Patients who mounted humoral immune responses had a longer progression-free survival (PFS) than those who did not (8.21 v 3.38 years; P =.018). Patients with Fc(gamma)RIIa 158 valine/valine (V/V) genotype also had a longer PFS than those with valine/phenylalanine (V/F) or phenylalanine/phenylalanine (F/F) genotypes (V/V, 8.21 v V/F, 3.38 years; P =.004; F/F, 4.47 years; P =.035). Multivariate analysis using the Cox proportional hazards model showed that V/V genotype and humoral immune responses were independent positive predictors for PFS. CONCLUSION: This study is the first to identify the predictive value of Fc(gamma)R polymorphism on clinical outcome in patients who received active immunotherapy with tumor antigen vaccines. Our results imply that the antibodies induced against a tumor antigen are beneficial and that Fc(gamma)R-bearing cells mediate an antitumor effect by killing antibody-coated tumor cells.

http://jcp.bmjjournals.com/cgi/content/abstract/58/1/83

DNA typing was requested to investigate a presumptive cancer diagnosis error by confirming whether benign and cancerous prostatic tissue in the same presurgical haematoxylin and eosin stained slide belonged to the same person. After independent histological re-examination of the slide by a pathologist, manual slide dissection was used to guarantee independent and high recovery DNA isolation from each tissue section, avoiding carryover and background contamination. Nuclear DNA quantification performed by real time polymerase chain reaction (PCR) revealed the absence of human DNA for short tandem repeat (STR) typing. Mitochondrial DNA was only obtained by performing PCR of very short fragments (~100 bp), indicating high DNA degradation. Different low frequency hypervariable region I haplotypes were obtained from each tissue section (normal tissue section haplotype: 16224C, 16234T, 16311C, 16356C; cancer tissue section haplotype: 16256T, 16270T, 16293G). Only the normal tissue section haplotype matched that obtained from the patient's blood sample, indicating that the cancer tissue section originated from an unknown patient. These results supported the hypothesis of sample mix up during block processing or slide preparation by a carryover mechanism. Mitochondrial genetic typing is recommended to exclude the possibility of carryover artefacts when low DNA content and high degradation compromise conventional STR typing.


http://jcp.bmjjournals.com/cgi/content/abstract/56/4/292

Background: Chronic neutrophilic leukaemia (CNL) is a rare myeloproliferative disorder of elderly patients characterised by sustained neutrophilia and splenomegaly. The diagnosis of CNL requires the exclusion of BCR/ABL positive chronic myelogenous leukaemia (CML) and of leukaemoid reactions (LRs). The differentiation between CNL and LR is problematic because both conditions share similar morphological features; it is also important because patients with CNL generally have a poor prognosis. Aims: To determine whether CNL and LR could be distinguished on the basis of different clonality patterns. Methods: Blood samples from 52 women were studied using the human androgen receptor gene assay (HUMARA). Results: Monoclarity was found in the neutrophils in all 17 patients with different myeloproliferative syndromes (MPSs), including those with CNL. In four of the patients with CNL, autologous T cells were also monoclonal, suggesting that they belonged to the neoplastic clone. This finding was in contrast to other MPSs in which T cells were almost always polyclonal. Of nine patients with clinically suspected LR, the neutrophils of five were polyclonal, whereas three patients had monoclonal neutrophils, suggesting that they might be in the process of developing an MPS. Among 26 healthy blood donors, 20 had polyclonal neutrophils and five showed skewed clonality patterns. One case of LR and one normal blood donor were scored "not informative" at the HUMARA locus. Conclusions: Clonality studies of blood neutrophils using HUMARA aid in distinguishing female patients with monoclonal CNL from those with LR. For the diagnosis of CNL, monoclonality of the neutrophils should be demonstrated whenever possible.

Aims: Use of the polymerase chain reaction for the detection of Mycobacterium tuberculosis (TB PCR) as a basis for making clinical decisions on the initiation of antituberculosis treatment was studied. Methods: A retrospective study involving a cohort of 155 patients being investigated for tuberculosis in an infectious disease consultation service was undertaken. TB PCR was performed on pulmonary and extrapulmonary specimens from these patients. The sensitivity of TB PCR was analysed. Results: Of the 155 patients, 144 fitted the clinical diagnosis of tuberculosis, and 112 of them were culture positive for M tuberculosis. Sixty (58.3%) patients with clinical features suggestive of tuberculosis received antituberculosis treatment based on positive TB PCR alone. Of 224 clinical specimens (138 pulmonary and 86 extrapulmonary) sent for TB PCR, 148 (99 pulmonary and 49 extrapulmonary) were positive in 117 patients. Of the 690 clinical specimens sent for culture, 279 were positive for M tuberculosis in 112 patients. The diagnostic sensitivity of TB PCR was 75.9% (85 of 112) and 81.3% (117 of 144) in patients with culture confirmed and clinically diagnosed tuberculosis, respectively. Using culture as the gold standard, the overall sensitivity of TB PCR was 78.3%, and for pulmonary and extrapulmonary specimens it was 82.3% and 72.0%, respectively. Conclusions: TB PCR is a rapid and reliable test in the diagnosis and management of tuberculosis.


Background: Low RNA yields from clinical samples are a limiting step for microarray technology. Aims: To design an accurate real time quantitative polymerase chain reaction (PCR) assay to assess the crucial step of global mRNA amplification performed before microarray hybridisation, using less than 1 {micro}g total RNA. Methods: Three RNA extraction procedures were compared for small size samples. Total RNA was amplified from universal RNA or the BC-H1 breast cancer micrometastatic cell line using three different protocols. Real time quantitative PCR technology was used for accurate measurement of urokinase plasminogen activator receptor and cytokeratin 8 RNA amplification rates and ratios, using primer sets binding at various distances from the 3' end of transcripts. A 50 mer oligomeric array targeting 87 genes potentially involved in breast cancer metastatic progression was built and hybridised with amplified RNA. Results: Eighteen nanograms of total RNA could be purified from 1000 BC-H1 micrometastatic cells. Amplification rates of 25 000 to 100 000 were achieved with as little as 10 ng of starting material. However, results were highly variable, depending on the amount of starting material, gene characteristics, sample quality, and protocols used. Oligomeric array hybridisation with 20 {micro}g reference RNA resulted in specific and reproducible signals for 83% of the genes, whereas mRNA amplification from less than 400 ng of starting material resulted in selective detection of signals from highly expressed genes. Conclusions: Improvements in the design of global mRNA amplification procedures and oligomeric arrays are needed to extract informative gene expression data from clinical samples containing limited cell numbers.

whether p53 mutations are associated with established risk factors for oral squamous cell carcinoma (OSCC). Methods: Fifty five OSCCs were investigated for p53 protein expression by immunohistochemistry (IHC). Ten of these cases, including five p53 immunopositive and five p53 immunonegative cases, were subjected to microdissection of representative tumour areas followed by sequence analysis for the detection of TP53 mutations. Results: Paired IHC and sequence analysis revealed that p53 immunopositivity in more than 25% of tumour cells was indicative of TP53 mutations, whereas p53 immunonegativity was not informative. Therefore, for p53 immunohistochemical interpretation, p53 immunonegative cases were excluded from the analysis and the cut off value for p53 immunopositivity was set at 25%. Of the OSCCs showing any p53 immunopositivity, 64% revealed staining in more than 25% of the tumour cells. p53 immunopositivity in more than 25% of the neoplastic cells was significantly associated with smoking but not with alcohol consumption. No significant association with smoking habits was found when OSCCs were dichotomised into p53 immunonegative and p53 immunopositive.

Conclusions: In OSCCs the following conclusions can be made: (1) p53 immunonegativity is not informative for TP53 mutations; (2) 25% p53 immunopositive cells appears to be a good cut off value to predict TP53 mutations; (3) p53 immunostaining patterns that appeared to be predictive for TP53 mutations were associated with the smoking habits of the patients.


http://jcp.bmjjournals.com/cgi/content/abstract/56/1/69

Aim: To determine the frequency of tumour budding and somatic APC mutation in a series of colorectal cancers stratified according to DNA microsatellite instability (MSI) status.

Material/Methods: Ninety five colorectal cancers were genotyped for APC mutation in the mutation cluster region (exon 15) and scored for the presence of tumour budding at the invasive margin in haematoxylin and eosin stained sections. A subset was immunostained for β-catenin and p16. Results: The frequency of both somatic APC mutation and tumour budding increased pari passu in cancers stratified as sporadic MSI high (MSI-H), hereditary non-polyposis colorectal cancer (HNPPCC), MSI low (MSI-L), and microsatellite stable (MSS). Both budding and APC mutation were significantly less frequent in sporadic MSI-H cancers than in MSI-L or MSS cancers. Tumour buds were characterised by increased immunostaining for both (beta) catenin and p16. Conclusion: Tumour budding is associated with an adverse prognosis. The lack of budding in MSI-H colorectal cancer may account for the improved prognosis of this subset and may be explained by an intact WNT signalling pathway and/or inactivated p16INK4a.


http://jcp.bmjjournals.com/cgi/content/abstract/56/1/36

Aims: To evaluate the chromosomal translocation t(11;18)(q21;q21) in gastrointestinal lymphomas. Methods: A possible API2-MLT fusion transcript specific to t(11;18)(q21;q21) was examined by means of reverse transcription-polymerase chain reaction (RT-PCR) in tumours from 47 cases of primary gastrointestinal lymphoma (28 low grade mucosa associated lymphoid tissue (MALT) lymphomas, four low grade MALT lymphomas with a high grade component, nine secondary diffuse large B cell lymphomas, four primary diffuse large B cell lymphomas, and two T cell lymphomas). Results: API2-MLT fusion was seen in four of 28 cases of low grade MALT lymphoma, but it was not seen in other types of lymphoma. Among the low grade MALT lymphomas, the fusion transcript was seen more frequently in colonic tumours than in gastric tumours (two of three compared with two of 24) and in tumours with submucosal invasion than in
those confined to the mucosa (four of 13 compared with 0 of 15). Helicobacter pylori negative tumours tended to show a higher positive rate than H pylori positive tumours (three of six compared with one of 21). None of the gastric tumours that responded to H pylori eradication expressed the API2-MLT fusion transcript. Conclusions: t(11;18)(q21;q21) seems to be one of the genetic alterations related to the development of gastrointestinal low grade MALT lymphoma. Such translocations may be predominantly associated with the development of intestinal MALT lymphoma.


http://jcp.bmjournals.com/cgi/content/abstract/57/7/766

Aims: To detect non-viral mRNA in human plasma that has been frozen for three years using a new protocol. Methods: Plasma from 15 patients with colorectal cancer and 10 normal subjects was separated and frozen with Trizol at -80{degrees}C for three years. As a control measure, plasma from 10 of the 15 patients was separated using the same protocol but no Trizol during storage. After three years, all samples were extracted using Trizol and RNeasy before the reverse transcriptase polymerase chain reaction was performed to detect non-viral (beta) catenin mRNA. In addition, extraction of three plasma samples by Trizol or RNeasy independently was carried out for comparison. Results: (beta) Catenin mRNA was detected in all 15 patient plasma samples and only one of the 10 normal subjects. In contrast, no (beta) catenin mRNA was found in the control and patient samples that were independently extracted by Trizol and RNeasy kit. Conclusions: This new protocol is a reliable method for extracting non-viral mRNA from the plasma of patients with cancer after longterm storage for three years. Extractions using Trizol and RNeasy kits independently could not isolate mRNA with sufficient quantity and quality for detection.


http://jcp.bmjournals.com/cgi/content/abstract/55/10/774

A study was carried out to compare the API20C technology with polymerase chain reaction amplification and direct sequencing of the short internal transcribed spacer region 2 (ITS2) for the identification of 58 isolates of invasive candida species obtained from patients with bloodstream infections over the seven year period 1994 to 2000. Overall, there was only one disagreement between the phenotypic and genotypic identification, where the API scheme identified the isolate as C albicans but the molecular method identified it as C dubliniensis. This study demonstrated that the API20C method is useful in the identification of Candida spp isolated from blood culture and that molecular methods do not enhance identifications made using the API20C scheme. However, for correct reporting of C dubliniensis, an emerging bloodborne pathogen, it is recommended that all isolates identified as C albicans by the API20C scheme are further examined phenotypically and/or genotypically.

Aim: To investigate the role of human papillomavirus (HPV) in the development of bladder transitional cell carcinoma (TCC). Methods: Seventy eight paraffin wax embedded TCC samples were tested for the presence of HPV by two methods. First, immunohistochemistry was carried out using a polyclonal antibody capable of detecting the capsid protein of all known papillomaviruses. The second method was a consensus GP5+/6+ primer mediated polymerase chain reaction (PCR) technique, with the products analysed by both agarose gel electrophoresis and an enzyme immunoassay using type specific oligonucleotide probes for 10 different mucosal genotypes. To exclude false negative results because of the poor quality of DNA extracted from paraffin wax embedded samples, the series was extended to include 20 further blocks for which the corresponding snap frozen unfixed tissue was available. Results: The two methods produced contrasting results, with 47 of the 78 samples positive for HPV antigen and none positive for HPV DNA. HPV DNA was not detected in the 20 additional paraffin wax embedded TCCs or in the 20 paired unfixed samples. In contrast, HPV DNA was amplified by PCR from all six of the paraffin wax embedded cervical carcinoma and anogenital wart control samples. Conclusion: The disparity between the two sets of results is probably caused by false positives resulting from the non-specificity of the polyclonal antibody used for immunohistochemistry. These results suggest that HPV is unlikely to play an aetiological role in the development of bladder TCC.


This report describes a hepatocellular carcinoma (HCC) with concomitant focal nodular hyperplasia (FNH) in a 56 year old Chinese man. There were two well circumscribed tumours measuring 3 x 2.5 x 2 cm and 2 x 1.5 x 1.5 cm. The larger mass was grey and soft with a small area of bleeding and necrosis and an intact capsule. The smaller mass was yellow and had no capsule. Clonal analysis was carried out to clarify the relation between the HCC and the adjacent FNH. The clonal analysis was based on the methylation pattern of the polymorphic X chromosome linked androgen receptor gene (HUMARA). In FNH, after HpaII digestion, the allelic bands showed two well defined peaks. The intensity of the two peaks in the DNA from cirrhotic tissue did not differ significantly, consistent with a random pattern of X chromosome inactivation. However, in HCC, after HpaII digestion, the allelic bands differed significantly in intensity. Therefore, there was a typical polyclonal pattern of inactivation in FNH but the HCC was interpreted as being monoclonal.

J. Clin. Pharmacol.  (1)


This study investigated 2 hypotheses about genotype-phenotype relationships for the efflux transporter, P-glycoprotein: (1) the presence of a synonymous C3435T variant in exon 26 of the
MDR1 gene correlates to higher plasma concentrations of a P-glycoprotein substrate, dicloxacillin, and (2) the effects of genotypic differences decrease under conditions of P-glycoprotein induction by rifampin. Eighteen healthy volunteers received two 1-g doses of dicloxacillin, one on the 1st study day and the other on the 11th day of rifampin dosing (600 mg daily). Dicloxacillin and its 5-hydroxymethyl metabolite were analyzed using liquid chromatography/tandem mass spectrometry. Mean dicloxacillin Cmax measurements were 30.5 {+/-} 13.5, 33.3 {+/-} 4.7, and 31.1 {+/-} 12.8 [micro]g/mL in individuals with the CC, CT, and TT genotype at position 3435 in exon 26 of the MDR1 gene. Following rifampin dosing, the mean dicloxacillin Cmax across genotypes decreased from 31.4 {+/-} 10.8 to 22.9 {+/-} 7.0 [micro]g/mL (P <.05), whereas the mean oral clearance increased from 235 {+/-} 82 to 297 {+/-} 71 mL/min (P <.001), and the mean absorption time increased from 0.71 {+/-} 0.55 to 1.34 {+/-} 0.77 h (P <.05). Rifampin treatment increased the formation clearance, Cmax, and AUC of the 5-hydroxymethyl metabolite by 135%, 119%, and 59%, respectively. The C3435T variant had no effect on dicloxacillin pharmacokinetics. The data suggested that rifampin induced intestinal P-glycoprotein and increased dicloxacillin metabolism.

J. Dent. Res.  (10)


http://jdr.iadrjournals.org/cgi/content/abstract/81/5/360

Tooth pulp contains steroid receptors and therefore is likely to respond to steroids. Steroids and cytokines together can alter steroid receptor content in many tissues; thus, similar mechanisms may exist in tooth pulp. In this study, reverse-transcription/polymerase chain-reaction was used to screen human pulp for the mRNAs encoding receptors for androgen (AR), estrogens (ER{beta}), and hepatocyte growth factor (HGF: c-Met). AR mRNA content was greater in male pulp vs. female pulp in all age groups. In both genders, AR mRNA content diminished with age. In pulp cell cultures, androstenedione, estradiol-17{beta}, and HGF each stimulated AR mRNA accumulation. Testosterone inhibited, whereas 5{alpha}-dihydrotestosterone did not affect, AR mRNA content. ER{beta} was not hormonally altered in pulp cell cultures. By showing steroid- and cytokine-orchestrated regulation of AR mRNA in vitro, it is possible that age- and/or pathogen-dependent changes in available steroids and cytokines can affect any androgen-responsiveness of pulp.


http://jdr.iadrjournals.org/cgi/content/abstract/83/8/639

Human herpesvirus-8 (HHV-8) is the etiologic agent of Kaposi's sarcoma (KS), which occurs in epidemic form in human immunodeficiency virus (HIV)-infected individuals. Saliva is the only mucosal fluid in which infectious HHV-8 has been identified, although factors associated with HHV-8 salivary shedding remain unclear. Our study performed PCR analysis for HHV-8 DNA in saliva (and other body fluids) in 66 HIV- and HHV-8-co-infected women without KS so that we could examine predictors for HHV-8 DNA detection. CD4 count was the most significant predictor
of HHV-8 salivary shedding, with increased prevalence of HHV-8 salivary DNA at higher CD4 counts. The odds of salivary HHV8 shedding at CD4 counts ≥ 350 cells/µL was 63 times the odds of shedding at CD4 < 350 (95%CI, 1.3-3078), with an increase in effect size when the analysis was restricted to those with a CD4 nadir > 200. Analysis of these data suggests an increased potential for HHV-8 transmission early in HIV infection, with implications for HHV-8 prevention.


http://jdr.iadrjournals.org/cgi/content/abstract/83/3/232

Colonization with Tannerella forsythensis may characterize the conversion of periodontally healthy sites into diseased sites. This three-year study describes the prevalence of T. forsythensis and its relationship to clinical loss of attachment (LOA) in a group of adolescents considered at risk of developing early chronic periodontitis. Adolescents with (LOA+) and without (LOA-) loss of attachment were examined at baseline and 1.5 and 3 yrs subsequently. On each occasion, attachment loss was measured on selected teeth, and the presence of T. forsythensis in their subgingival plaque samples was determined by PCR. T. forsythensis prevalence in LOA+ subjects at baseline (64%) increased to 82% and 86% on subsequent examinations. In contrast, prevalence of T. forsythensis in LOA- subjects was always significantly lower (25%, 36%, and 32%, respectively). The odds of loss of attachment were 8.16 times greater in subjects infected with T. forsythensis at each examination. These results suggest that T. forsythensis is strongly associated with loss of attachment in this adolescent population.


http://jdr.iadrjournals.org/cgi/content/abstract/81/11/738

Amelogenesis imperfecta (AI) is currently classified into 14 distinct subtypes based on various phenotypic criteria; however, the gene responsible for each phenotype has not been defined. We performed molecular genetic studies on a Japanese family with a possible autosomal-dominant form of AI. Previous studies have mapped an autosomal-dominant human AI locus to chromosome 4q11-q21, where two candidate genes, ameloblastin and enamelin, are located. We studied AI patients in this family, focusing on these genes, and found a mutation in the enamelin gene. The mutation detected was a heterozygous, single-G deletion within a series of 7 G residues at the exon 9-intron 9 boundary of the enamelin gene. The mutation was detected only in AI patients in the family and was not detected in other unaffected family members or control individuals. The male proband and his brother showed hypoplastic enamel in both their deciduous and permanent teeth, and their father showed local hypoplastic defects in the enamel of his permanent teeth. The clinical phenotype of these patients is similar to that of the first report of AI caused by an enamelin gene mutation. Thus, heterogeneous mutations in the enamelin gene are responsible for an autosomal-dominant hypoplastic form of AI.

Tooth development is under strict genetic control. Oligodontia is defined as the congenital absence of 6 or more permanent teeth, excluding the third molar. The occurrence of non-syndromic oligodontia is poorly understood, but in recent years several cases have been described where a single gene mutation is associated with oligodontia. Several studies have shown that MSX1 and PAX9 play a role in early tooth development. We screened one family with non-syndromic oligodontia for mutations in MSX1 and PAX9. The pedigree showed an autosomal-dominant pattern of inheritance. Direct sequencing and restriction enzyme analysis revealed a novel heterozygous A to G transition mutation in the AUG initiation codon of PAX9 in exon 1 in the affected members of the family. This is the first mutation found in the initiation codon of PAX9, and we suggest that it causes haploinsufficiency.


A series of reports has revealed that adenosine has a plethora of biological actions toward a large variety of cells. In this study, we investigated the influence of adenosine receptor activation on iNOS mRNA expression in human gingival epithelial cells (HGEC) and SV-40-transformed HGEC. HGEC expressed adenosine receptor subtypes A1, A2a, and A2b, but not A3 mRNA. Ligation of adenosine receptors by a receptor agonist, 2-chloroadenosine (2CADO), enhanced iNOS mRNA expression by both HGEC and transformed HGEC. In addition, the adenosine receptor agonist enhanced the production of NO2-/NO3-, NO-derived stable end-products. An enhanced expression of iNOS mRNA and NO2-/NO3- was also observed when SV40-transformed HGEC were stimulated with CPA or CGS21680, A1- or A2a-selective adenosine receptor agonists, respectively. These results provide new evidence for the possible involvement of adenosine in the regulation of inflammatory responses by HGEC in periodontal tissues.


A blood isolate of Streptococcus mutans strain TW871 shows relatively low homology with MT8148, a reference oral isolate strain, and lacks the serotype-specific polysaccharide antigen, suggesting that other cell-surface structures correlate with cariogenicity. We compared cariogenicity of TW871 with MT8148 (serotype c) and blood isolate TW964 (serotype f) in rats. Strain TW871 showed significantly lower cariogenicity than MT8148 or TW964 and expressed significantly lower sucrose-independent cellular adhesion to saliva-coated hydroxyapatite and dextran-binding activity than strain MT8148. Strains TW871 and TW964 showed a defect in the gbpA gene by Southern hybridization analysis, while sequencing analysis revealed gbpC variation in TW871. These results suggest that variation in GbpC may alter cellular adherence properties and can be correlated with the cariogenicity of S. mutans in this strain.

Glucosyltransferases (GTF)-I and GTF-SI of Streptococcus mutans synthesize water-insoluble and both water-soluble and -insoluble glucans, respectively, and play essential roles in the sucrose-dependent adhesion of the organism to tooth surfaces. To examine the interactions of different GTFs on artificial biofilm formed by S. mutans and other oral streptococci, we generated GTF-I- and GTF-SI-hyperproducing isogenic mutant strains. Transformant B42-21, which hyperexpressed GTF-SI, exhibited firm adhesion in the presence of sucrose, whereas transformant B42-10, which hyperexpressed GTF-I, failed to exhibit firm adhesion. Furthermore, co-culture of transformant B42-21 with water-soluble glucan-synthesizing Streptococcus sanguinis yielded firm adhesion, while the addition of dextran T10 to B42-21 growing culture had no effect on adhesion. These findings suggest that GTF-SI has a strong effect on sucrose-dependent adhesion and is essential for biofilm formation on smooth surfaces, in cooperation with water-soluble glucans synthesized de novo by oral streptococci that inherently lack cell adhesion ability.


A novel inhibitor of apoptosis, survivin, plays a role in oncogenesis. To determine the potential involvement of survivin in oral carcinogenesis, we investigated the distribution of survivin protein expression in oral squamous cell carcinomas (OSCCs) and oral pre-malignant lesions. The mRNA expression level and methylation status of the gene also were evaluated in OSCCs and OSCC-derived cell lines. In immunohistochemistry, 58% of tumors and 37% of pre-malignant lesions examined were positive for survivin, while no immunoreaction was observed in corresponding normal tissues. The reverse-transcription/polymerase chain-reaction revealed similar changes in survivin gene expression levels. Furthermore, of the 9 normal oral tissues with no survivin gene expression, 4 showed methylation of the gene, while no methylation was detected in the corresponding tumorous tissues. The results suggest that survivin plays an important role during oral carcinogenesis, and that the gene expression may be regulated by an epigenetic mechanism.


Colony-stimulating factor-one (CSF-1) and parathyroid-hormone-related protein (PTHrP) down-regulate osteoprotegerin (OPG) gene expression in the dental follicle of the rat first mandibular molar. To examine this regulation at the signal transduction level, we treated cultured dental follicle cells with either phorbolmyristate acetate (PMA) or dibutryl cyclic AMP (dbcAMP) to activate either protein kinase C (PKC) or protein kinase A (PKA). Our results demonstrate that PMA up-regulates OPG gene expression and down-regulates the expression of CSF-1 and the PTHrP receptor (PTHrP-R). Conversely, dbcAMP down-regulates OPG expression and up-regulates CSF-1 and PTHrP-R expression. Immunostaining shows that PMA also increases the steady-state levels of protein. Thus, treatment with agents that affect protein kinase activity also enhance the steady-state mRNA and protein levels of OPG, as well as decreasing the mRNA levels of CSF-1 and PTHrP-R. The PKC-{alpha} isoform may be critical in OPG regulation because PKC-{alpha} gene expression is enhanced by PMA and reduced by either CSF-1 or
PTHrP.

J. Endocrinol. (2)


http://joe.endocrinology-journals.org/cgi/content/abstract/183/1/183

Vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of diabetic nephropathy. We investigated serial changes of VEGF in the kidney and assessed whether glomerular and urinary VEGF levels are related to the severity of diabetic nephropathy. Furthermore, we examined the relationship between urinary VEGF levels and the urinary albumin excretion (UAE) rate in Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats. Glomerular VEGF mRNA expression and protein synthesis were evaluated by the reverse transcription-polymerase chain reaction, immunohistochemical staining and in situ hybridization. Urinary levels of VEGF were determined by enzyme-linked immunosorbent assay. UAE was significantly higher in OLETF rats than in control Long-Evans-Tokushima-Fatty (LETO) rats throughout the study period. Urinary VEGF levels were significantly higher from 25 to 37 weeks, and then gradually reduced until 55 weeks, although the levels were still higher than those in control rats. Urinary VEGF levels also showed a significant positive correlation with UAE (r=0.262, P=0.045) and serum creatinine (r=0.398, P=0.044), and were found to be independently correlated with UAE by Spearman's rank correlation. By immunohistochemical staining and in situ hybridization, VEGF was mainly detected in the podocytes in the glomeruli. Interestingly, a significant increase in VEGF mRNA expression was observed in the early period of diabetic nephropathy, and this was associated with increased urinary VEGF excretion. Thus, the overproduction of VEGF in the diabetic kidney may participate in the pathogenesis of early-stage diabetic nephropathy.


http://joe.endocrinology-journals.org/cgi/content/abstract/183/2/257

Medullary thyroid carcinoma (MTC) occurs as a sporadic form (75%) or as an autosomal dominant inherited familial disorder (25%) called familial MTC (FMTC) or as multiple endocrine neoplasia type 2 (MEN2) syndromes. Germ-line mutations in the rearranged during transfection (RET) proto-oncogene in exons 10, 11, 13, 14, 15 and 16 are known to be a cause of most of the familial forms. In this paper we report molecular genetic testing of 106 families with MTC (358 tested persons) from the Czech Republic in which we directly sequenced these six exons of the RET proto-oncogene. We detected germ-line mutations in 100% of MEN2B families (4/4 families), 90% of MEN2A families (9/10), 40% of FMTC families (4/10) and 7% of apparently sporadic MTC (6/82). Eleven different germ-line mutations were revealed. MEN2B was associated with mutation Met918 Thr in exon 16. In one MEN2B family beside this mutation the Tyr791 Phe was also found, which has not yet been reported. MEN2A was restricted to different mutations in exon 11 (codon 634). In FMTC and sporadic MTC families the mutations in exons 10, 11, 13 and 14 were detected. The genotype/phenotype correlations are given. Genetic testing revealed germ-line mutations in 23 index patients, 24 family members and excluded them in 53 relatives.

http://jeq.scijournals.org/cgi/content/abstract/32/1/47

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide) is a cyclodiene organochlorine currently used as an insecticide all over the world and its residues are posing a serious environmental threat. This study reports the isolation and identification of enriched microorganisms, capable of degrading endosulfan. Enrichment was achieved by using the insecticide as either the sole source of carbon or sulfur in parallel studies. Two strains each of fungi (F1 and F4) and bacteria (BF2 and B4) were selected using endosulfan as a sole carbon source. A Pandoraea species (Lin-3) previously isolated in our laboratory using lindane (γ-HCH) as a carbon source was also screened for endosulfan degradation. F1 and F4 (Fusarium ventricosum) degraded α-endosulfan by as much as 82.2 and 91.1% and β-endosulfan by 78.5 and 89.9%, respectively, within 15 d of incubation. Bacterial strains B4 and Lin-3 degraded α-endosulfan up to 79.6 and 81.8% and β-endosulfan up to 83.9 and 86.8%, respectively, in 15 d. Among the bacterial strains isolated by providing endosulfan as a sulfur source, B4s and F4t degraded α-endosulfan by as much as 70.4 and 68.5% and β-endosulfan by 70.4 and 70.8%, respectively, after 15 d. Degradation of the insecticide occurred concomitant with bacterial growth reaching an optical density (OD600) of 0.366 and 0.322 for B4 and Lin-3, respectively. High OD600 was also noted with the other bacterial strains utilizing endosulfan as a sulfur source. Fungal and bacterial strains significantly decreased the pH of the nutrient culture media while growing on endosulfan. The results of this study suggest that these novel strains are a valuable source of potent endosulfan-degrading enzymes for use in enzymatic bioremediation.


http://jeb.biologists.org/cgi/content/abstract/207/22/3865

Increased skeletal muscle capillary density would be a logical adaptive mechanism to chronic hypoxic exposure. However, animal studies have yielded conflicting results, and human studies are sparse. Neoformation of capillaries is dependent on endothelial growth factors such as vascular endothelial growth factor (VEGF), a known target gene for hypoxia inducible factor 1 (HIF-1). We hypothesised that prolonged exposure to high altitude increases muscle capillary density and that this can be explained by an enhanced HIF-1α expression inducing an increase in VEGF expression. We measured mRNA levels and capillary density in muscle
biopsies from vastus lateralis obtained in sea level residents (SLR; N=8) before and after 2 and 8 weeks of exposure to 4100 m altitude and in Bolivian Aymara high-altitude natives exposed to approximately 4100 m altitude (HAN; N=7). The expression of HIF-1{alpha} or VEGF mRNA was not changed with prolonged hypoxic exposure in SLR, and both genes were similarly expressed in SLR and HAN. In SLR, whole body mass, mean muscle fibre area and capillary to muscle fibre ratio remained unchanged during acclimatization. The capillary to fibre ratio was lower in HAN than in SLR (2.4{+/-}0.1 vs 3.6{+/-}0.2; P<0.05). In conclusion, human muscle VEGF mRNA expression and capillary density are not significantly increased by 8 weeks of exposure to high altitude and are not increased in Aymara high-altitude natives compared with sea level residents.


We have previously described the first cloning and partial characterization of carbonic anhydrase from larval Aedes aegypti mosquitoes. Larval mosquitoes utilize an alkaline digestive environment in the lumen of their anterior midgut, and we have also demonstrated a critical link between alkalization of the gut and carbonic anhydrase(s). In this report we further examine the nature of the previously described carbonic anhydrase and test the hypothesis that its pattern of expression is consistent with a role in gut alkalization. Additionally we take advantage of the recently published genome of the mosquito Anopheles gambiae to assess the complexity of the carbonic anhydrase gene family in these insects. We report here that the previously described carbonic anhydrase from Aedes aegypti is similar to mammalian CA IV in that it is a GPI-linked peripheral membrane protein. In situ hybridization analyses identify multiple locations of carbonic anhydrase expression in the larval mosquito. An antibody prepared against a peptide sequence specific to the Aedes aegypti GPI-linked carbonic anhydrase labels plasma membranes of a number of cell types including neuronal cells and muscles. A previously undescribed subset of gut muscles is specifically identified by carbonic anhydrase immunohistochemistry. Bioinformatic analyses using the Ensembl automatic analysis pipeline show that there are at least 14 carbonic anhydrase genes in the Anopheles gambiae genome, including a homologue to the GPI-linked gene product described herein. Therefore, as in mammals which similarly possess numerous carbonic anhydrase genes, insects require a large family of these genes to handle the complex metabolic pathways influenced by carbonic anhydrases and their substrates.


The heat shock proteins (Hsps) play a positive role in lifespan determination, and histone acetylation has been shown to be involved in transcription of hsp genes in Drosophila. To further determine if hsp22 and hsp70 expression is correlated with lifespan, and if histone acetylation participates in this process, RNA levels for hsp22 and hsp70 were analyzed throughout the lifespan in the long-lived and short-lived iso-female lines. The results showed that hsp22 and hsp70 RNA levels were higher in long-lived line than in short-lived line and that the long-lived flies responded more rapidly to heat but were more tolerant to high temperature. Moreover, we investigated the influence of histone acetylation modification on longevity and on hsp gene expression by using histone deacetylase (HDAC) inhibitors TSA and BuA. The results demonstrated that both inhibitors were able to extend the lifespan and promote hsp22 and hsp70 expression. However, the optimal concentrations of these inhibitors, and probably the mechanisms of their actions, vary with the genetic background. In addition, we showed that
HDAC inhibitors caused the hyperacetylation of core histone H3, implicating the involvement of chromatin modulation in hsp gene transcription. These data suggested a close correlation among histone acetylation, hsp gene expression and longevity in D. melanogaster.


http://jxb.oupjournals.org/cgi/content/abstract/54/392/2439

The ascorbate content declined rapidly in broccoli (Brassica oleracea L. var. italica) florets, but not in the stem tissue, during post-harvest senescence. Ascorbate peroxidase (APX), ascorbate oxidase (AO), L-galactono-1,4-lactone dehydrogenase (GLDH), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) were investigated in gene expression after harvest in both florets and the stem tissue of broccoli. Cytosolic gene expressions (BO-APX 1, BO-APX 2, BO-AO, BO-MDAR 2, and BO-GR) were stimulated actively in broccoli florets after harvest. By contrast, it was observed that mRNA levels of chloroplastic APX, BO-sAPX and BO-tbAPX, had decreased by 12 h after harvest in broccoli florets, suggesting that the active oxygen species (AOS) scavenging system in chloroplasts was largely abolished in florets during the early hours of the post-harvest period. In addition, gene expressions in GLDH and other chloroplastic enzymes such as BO-MDAR 1 and BO-DHAR decreased rapidly within 24 h after harvest. Ethylene treatment had no effect on the ascorbate level and the expression of all genes investigated. The expressions of BO-GLDH and chloroplastic genes (BO-sAPX, BO-tbAPX, BO-MDAR 1, and BO-DHAR) mRNA were suppressed by treatment with methyl jasmonate (MJ) and abscisic acid (ABA) and were accompanied by the acceleration of ascorbate degradation. These data suggest that ascorbate metabolism tends to be inactivated in chloroplasts by transcriptional regulation, but not in the cytosol, when ascorbate decreases under stress conditions.


http://jxb.oupjournals.org/cgi/content/abstract/56/409/65

The relationship between sucrose (Suc) and ascorbate (AA) metabolism was investigated in harvested broccoli (Brassica oleracea L. var. italica) florets. Decreases in both Suc and AA content were observed in broccoli florets 48 h after all the leaves were excised, but none were observed when the plants were kept intact or with leaves attached in a room at 20 °C. In harvested broccoli plants without leaves and roots, continuous absorption of a 10% (w/v) Suc solution from the cut surface of the stem suppressed the degreening of sepals and the loss of AA content in florets. The expression of the genes related to AA metabolism in chloroplasts and its biosynthesis were up-regulated by Suc feeding in broccoli florets. These data suggest that a decline in Suc leads to considerable damage not only to AA biosynthesis but also to the hydrogen peroxide-scavenging system in chloroplasts. In addition, the cessation of the Suc supply from leaves can be the main factor of AA degradation in harvested broccoli florets.

http://jxb.oupjournals.org/cgi/content/abstract/56/414/1093

The effects of increasing light and of a heterologous bHLH transcription factor on the accumulation of condensed tannins (CT) were investigated in leaves of Lotus corniculatus, a model legume species which accumulates these secondary metabolites in leaves as well as reproductive tissues. Light and expression of the transgene increased the level of CT in a synergistic way. To monitor how the changes in accumulation of condensed tannins were achieved, the level of expression of four key genes in the flavonoid pathway was estimated by real-time RT-PCR analysis. Early genes of the pathway (PAL and CHS) were affected less in their expression and so appeared to be less involved in influencing the final level of CT than later genes in the pathway (DFR and ANS). Steady-state levels of DFR and ANS transcripts showed a strong positive correlation with CT and these genes might be considered the first rate-limiting steps in CT biosynthesis in Lotus leaves. However, additional factors mediated by light are limiting CT accumulation once these genes are up-regulated by the transgene. Therefore, the increment of the steady-state mRNA level for DFR and ANS might not be sufficient to up-regulate condensed tannins in leaves. The real-time RT-PCR approach adopted showed that members within the CHS and DFR gene families are differentially regulated by the exogenous bHLH gene and light. This finding is discussed in relation to the approaches for controlling CT biosynthesis and for studying the expression profile of multi-gene families.


http://jxb.oupjournals.org/cgi/content/abstract/55/406/2251

Sorghum [Sorghum bicolor (L.) Moench] roots exude a potent bioherbicide known as sorgoleone, which is produced in living root hairs and is phytotoxic to broadleaf and grass weeds at concentrations as low as 10 {micro}M. Differential gene expression was studied in sorghum (S. bicolorxS. sudanense) cv. SX17 between roots with abundant root hairs and those without root hairs using a modified differential display approach. A differentially expressed gene, named SOR1, was cloned by using Rapid Amplification of the 5' ends of cDNA (5'-RACE). Real-time PCR analysis of multiple tissues of sorghum SX17 revealed that the SOR1 transcript level in root hairs was more than 1000 times higher than that of other tissues evaluated, including immature leaf, mature leaf, mature stem, panicle, and roots with hairs removed. Semi-quantitative RT-PCR revealed that SOR1 was expressed in the sorgoleone-producing roots of sorghum SX17, shattercane [S. bicolor (L.) Moench], and johnsongrass [S. halepense (L.) Pers.], but not in the shoots of sorghum or in the roots of sweet corn (Zea mays L.) Summer Flavor 64Y*, in which sorgoleone production was not detected by HPLC analysis. Similarity searches indicated that SOR1 probably encodes a novel desaturase, which might be involved in the formation of a unique and specific double bonding pattern within the long hydrocarbon tail of sorgoleone.
We report the identification of two novel minor histocompatibility antigens (mHAgs), encoded by two separate single nucleotide polymorphisms on a single gene, BCL2A1, and restricted by human histocompatibility leukocyte antigen (HLA)-A*2402 (the most common HLA-A allele in Japanese) and B*4403, respectively. Two cytotoxic T lymphocyte (CTL) clones specific for these mHAgs were first isolated from two distinct recipients after hematopoietic cell transplantation. Both clones lyse only normal and malignant cells within the hematopoietic lineage. To localize the gene encoding the mHAgs, two-point linkage analysis was performed on the CTL lytic patterns of restricting HLA-transfected B lymphoblastoid cell lines obtained from Centre d'Etude du Polymorphisme Humain. Both CTL clones showed a completely identical lytic pattern for 4 pedigrees and the gene was localized within a 3.6-cM interval of 15q24.3-25.1 region that encodes at least 46 genes. Of those, only BCL2A1 has been reported to be expressed in hematopoietic cells and possess three nonsynonymous nucleotide changes. Minigene transfection and epitope reconstitution assays with synthetic peptides identified both HLA-A*2402- and B*4403-restricted mHAg epitopes to be encoded by distinct polymorphisms within BCL2A1.

Class switch recombination (CSR), similar to V(D)J recombination, is thought to involve DNA double strand breaks and repair by the nonhomologous end-joining pathway. A key component of this pathway is DNA-dependent protein kinase (DNA-PK), consisting of a catalytic subunit (DNA-PKcs) and a DNA-binding heterodimer (Ku70/80). To test whether DNA-PKcs activity is essential for CSR, we examined whether IgM+ B cells from scid mice with site-directed H and L chain transgenes were able to undergo CSR. Although B cells from these mice were shown to lack DNA-PKcs activity, they were able to switch from IgM to IgG or IgA with close to the same efficiency as B cells from control transgenic and nontransgenic scid/+ mice, heterozygous for the scid mutation. We conclude that CSR, unlike V(D)J recombination, can readily occur in the absence of DNA-PKcs activity. We suggest nonhomologous end joining may not be the (primary or only) mechanism used to repair DNA breaks during CSR.

Human natural killer (NK) cells express a series of activating receptors and coreceptors that are involved in recognition and killing of target cells. In this study, in an attempt to identify the cellular ligands for such triggering surface molecules, mice were immunized with NK-susceptible target cells. On the basis of a functional screening, four mAbs were selected that induced a partial...
down-regulation of the NK-mediated cytotoxicity against the immunizing target cells. As revealed by biochemical analysis, three of such mAbs recognized molecules of \([\sim]70\) kD. The other mAb reacted with two distinct molecules of \([\sim]65\) and 60 kD, respectively. Protein purification followed by tryptic digestion and mass spectra analysis, allowed the identification of the 70 kD and the 65/60 kD molecules as PVR (CD155) and Nectin-2 {delta}/\{alpha\} (CD112), respectively. PVR-Fc and Nectin-2-Fc soluble hybrid molecules brightly stained COS-7 cells transfected with the DNAM-1 (CD226) construct, thus providing direct evidence that both PVR and Nectin-2 represent specific ligands for the DNAM-1 triggering receptor. Finally, the surface expression of PVR or Nectin-2 in cell transfectants resulted in DNAM-1-dependent enhancement of NK-mediated lysis of these target cells. This lysis was inhibited or even virtually abrogated upon mAb-mediated masking of DNAM-1 (on NK cells) or PVR or Nectin-2 ligands (on cell transfectants).


http://www.jem.org/cgi/content/abstract/197/9/1183

IgG1, IgG2a, and IgG2b, passively administered with soluble Ags, enhance specific Ab responses. The effect of IgG3 in this type of feedback regulation has not been studied previously. We immunized mice with trinitrophenyl (TNP)-coupled carrier proteins (bovine serum albumin [BSA] or ovalbumin [OVA]) alone or complexed to monoclonal TNP-specific IgG3. The carrier-specific Ab responses were enhanced by several hundred-fold by IgG3. Enhancement was significantly impaired in mice depleted of complement factor C3 and in mice lacking complement receptors 1 and 2 (Cr2-/-). In contrast, mice lacking the common Fc-receptor gamma chain (FcR\{gamma\}-/-), resulting in reduced expression of Fc\{gamma\}RI and lack of Fc\{gamma\}RII, and mice lacking Fc\{gamma\}RIIB (Fc\{gamma\}RIIB-/-), responded equally well to immunization with IgG3-complexed Ag as wild-type controls. These findings demonstrate that IgG3 can induce feedback enhancement and that IgG3, in analogy with IgM, uses the complement system for this function.


http://www.jem.org/cgi/content/abstract/195/5/617

The important role played by CD8+ T lymphocytes in the control of parasitic and viral infections, as well as tumor development, has raised the need for the development of adjuvants capable of enhancing cell-mediated immunity. It is well established that protective immunity against liver stages of malaria parasites is primarily mediated by CD8+ T cells in mice. Activation of natural killer T (NKT) cells by the glycolipid ligand, \{alpha\}-galactosylceramide ((\alpha\)-GalCer), causes bystander activation of NK, B, CD4+, and CD8+ T cells. Our study shows that coadministration of \{alpha\}-GalCer with suboptimal doses of irradiated sporozoites or recombinant viruses expressing a malaria antigen greatly enhances the level of protective anti-malaria immunity in mice. We also show that coadministration of \{alpha\}-GalCer with various different immunogens strongly enhances antigen-specific CD8+ T cell responses, and to a lesser degree, Th1-type responses. The adjuvant effects of \{alpha\}-GalCer require CD1d molecules, \{V\}\{alpha\}14 NKT cells, and interferon \{gamma\}. As \{alpha\}-GalCer stimulates both human and murine NKT cells, these findings should contribute to the design of more effective vaccines against malaria and other intracellular pathogens, as well as tumors.

http://www.jem.org/cgi/content/abstract/197/9/1125

Amongst the many ploys used by microbial pathogens to interfere with host immune responses is the production of proteins with the properties of superantigens. These properties enable superantigens to interact with conserved variable region framework subdomains of the antigen receptors of lymphocytes rather than the complementarity determining region involved in the binding of conventional antigens. To understand how a B cell superantigen affects the host immune system, we infused protein A of Staphylococcus aureus (SpA) and followed the fate of peripheral B cells expressing B cell receptors (BCRs) with VH regions capable of binding SpA. Within hours, a sequence of events was initiated in SpA-binding splenic B cells, with rapid down-regulation of BCRs and coreceptors, CD19 and CD21, the induction of an activation phenotype, and limited rounds of proliferation. Apoptosis followed through a process heralded by the dissipation of mitochondrial membrane potential, the induction of the caspase pathway, and DNA fragmentation. After exposure, B cell apoptotic bodies were deposited in the spleen, lymph nodes, and Peyer's patches. Although in vivo apoptosis did not require the Fas death receptor, B cells were protected by interleukin (IL)-4 or CD40L, or overexpression of Bcl-2. These studies define a pathway for BCR-mediated programmed cell death that is VH region targeted by a superantigen.


http://www.jem.org/cgi/content/abstract/197/3/333

In the absence of thymopoiesis, T lymphocytes are nevertheless present, mainly in the gut epithelium. Ontogeny of the extrathymic pathway and the extent of its involvement in euthymic mice are controversial. These questions have been addressed by assessing the expression of recombinase activating gene (RAG) through the use of green fluorescent protein RAG2 transgenic mouse models. In athymic mice, T lymphopoiesis occurs mainly in the mesenteric lymph node and less in the Peyer's patches. Ontogenic steps of this lymphopoiesis resemble those of thymopoiesis, but with an apparent bias toward {gamma}{delta} T cell production and with a paucity of oligoclonal {alpha}{beta} T cells possibly resulting from a deficit in positive selection. Whether in athymic or euthymic mice, neither T intraepithelial lymphocytes (IEL) nor cryptopatch cells (reported to contain precursors of IEL) displayed fluorescence indicating recent RAG protein synthesis. Newly made T cells migrate from the mesenteric node into the thoracic duct lymph to reach the gut mucosa. In euthymic mice, this extrathymic pathway is totally repressed, except in conditions of severe lymphocytic depletion. Thus, in normal animals, all gut T IEL, including CD8(alpha){alpha}+ cells, are of thymic origin, CD8(alpha){alpha}+ TCR(alpha){beta}+ IEL being the likely progeny of double negative NK1-1- thymocytes, which show polyclonal V(alpha) and V(beta) repertoires.

Although Toll-like receptors (TLRs) are critical mediators of the immune response to pathogens, the influence of polymorphisms in this gene family on human susceptibility to infection is poorly understood. We demonstrated recently that TLR5 recognizes flagellin, a potent inflammatory stimulus present in the flagellar structure of many bacteria. Here, we show that a common stop codon polymorphism in the ligand-binding domain of TLR5 (TLR5392STOP) is unable to mediate flagellin signaling, acts in a dominant fashion, and is associated with susceptibility to pneumonia caused by Legionella pneumophila, a flagellated bacterium. We also show that flagellin is a principal stimulant of proinflammatory cytokine production in lung epithelial cells. Together, these observations suggest that TLR5392STOP increases human susceptibility to infection through an unusual dominant mechanism that compromises TLR5's essential role as a regulator of the lung epithelial innate immune response.


Control of infection with virulent Mycobacterium tuberculosis (Mt) in mice is dependent on the generation of T helper (Th)1-mediated immunity that serves, via secretion of interferon (IFN)-\(\gamma\) and other cytokines, to upregulate the antitycobacterial function of macrophages of which the synthesis of inducible nitric oxide synthase (NOS)2 is an essential event. As a means to understanding the basis of Mt virulence, the ability of gene-deleted mice incapable of making NOS2 (NOS2-/-), gp91Phox subunit of the respiratory burst NADPH-oxidase complex (Phox-/-), or either enzyme (NOS2/Phox-/-), to control airborne infection with the avirulent R1Rv and H37Ra strains of Mt was compared with their ability control infection with the virulent H37Rv strain. NOS2-/-, Phox-/-, and NOS2/Phox-/- mice showed no deficiency in ability to control infection with either strain of avirulent Mt. By contrast, NOS2-/- mice, but not Phox-/- mice, were incapable of controlling H37Rv infection and died early from neutrophil-dominated lung pathology. Control of infection with avirulent, as well as virulent Mt, depended on the synthesis of IFN-\(\gamma\), and was associated with a substantial increase in the synthesis in the lungs of mRNA for IFN-\(\gamma\) and NOS2, and with production of NOS2 by macrophages at sites of infection. The results indicate that virulent, but not avirulent, Mt can overcome the growth inhibitory action of a Th1-dependent, NOS2-independent mechanism of defense.


Interferon (IFN)-\(\gamma\)-producing CD8+ T cells are important for the successful resolution of the obligate intracellular parasite Toxoplasma gondii by preventing the reactivation or controlling a repeat infection. Previous reports from our laboratory have shown that exogenous interleukin (IL)-15 treatment augments the CD8+ T cell response against the parasite. However, the role of endogenous IL-15 in the proliferation of activated/memory CD8+ T cells during toxoplasma or any other infection is unknown. In this study, we treated T. gondii immune mice with soluble IL-15 receptor \(\alpha\) (sIL-15R\(\alpha\)) to block the host endogenous IL-15. The treatment markedly reduced the ability of the immune animals to control a lethal infection. CD8+ T cell activities in the
sIL-15R{alpha}-administered mice were severely reduced as determined by IFN-{gamma} release and target cell lysis assays. The loss of CD8+ T cell immunity due to sIL-15R{alpha} treatment was further demonstrated by adoptive transfer experiments. Naive recipients transferred with CD44hi activated/memory CD8+ T cells and treated with sIL-15R{alpha} failed to resist a lethal T. gondii infection. Moreover, sIL-15R{alpha} treatment of the recipients blocked the ability of donor CD44hi activated/memory CD8+ T cells to replicate in response to T. gondii challenge. To our knowledge, this is the first demonstration of the important role of host IL-15 in the development of antigen-specific memory CD8+ T cells against an intracellular infection.


http://www.jem.org/cgi/content/abstract/198/4/635

Recent studies suggest that DNA polymerase \( \eta \) (pol{eta}) and DNA polymerase \( \iota \) (pol{ita}) are involved in somatic hypermutation of immunoglobulin variable genes. To test the role of pol{ita} in generating mutations in an animal model, we first characterized the biochemical properties of murine pol{ita}. Like its human counterpart, murine pol{ita} is extremely error-prone when catalyzing synthesis on a variety of DNA templates in vitro. Interestingly, when filling in a 1 base-pair gap, DNA synthesis and subsequent strand displacement was greatest in the presence of both pols {ita} and {eta}. Genomic sequence analysis of Poli led to the serendipitous discovery that 129-derived strains of mice have a nonsense codon mutation in exon 2 that abrogates production of pol{ita}. Analysis of hypermutation in variable genes from 129/SvJ (Poli-/-) and C57BL/6J (Poli+/+) mice revealed that the overall frequency and spectrum of mutation were normal in pol{ita}-deficient mice. Thus, either pol{ita} does not participate in hypermutation, or its role is nonessential and can be readily assumed by another low-fidelity polymerase.


http://www.jem.org/cgi/content/abstract/197/3/281

Here it is shown that the phenotype of adult mice lacking the first enhancer (DNA hypersensitive site I) and the distal promoter of the GATA-1 gene (neo{Delta}HS or GATA-1low mutants) reveals defects in mast cell development. These include the presence of morphologically abnormal alcian blue+ mast cells and apoptotic metachromatic mast cell precursors in connective tissues and peritoneal lavage and numerous (60-70% of all the progenitors) "unique" trilineage cells committed to erythroid, megakaryocytic, and mast pathways in the bone marrow and spleen. These abnormalities, which were mirrored by impaired mast differentiation in vitro, were reversed by retroviral-mediated expression of GATA-1 cDNA. These data indicate an essential role for GATA-1 in mast cell differentiation.


http://www.jem.org/cgi/content/abstract/200/11/1407
Human immunodeficiency virus (HIV)-specific CD8+ T cells persist in high frequencies in HIV-infected patients despite impaired CD4+ T helper response to the virus, but, unlike other differentiated effector cytotoxic T lymphocytes, most continue to express the tumor necrosis factor receptor family member CD27. Because the ligand for CD27 (CD70) is also overexpressed in HIV-infected hosts, we examined the nature of expression and potential functional consequences of CD27 expression on HIV-specific CD8+ T cells. Analysis of CD27+ and CD27- T cells derived from the same HIV-specific clone revealed that retention of CD27 did not interfere with acquisition of effector functions, and that after T cell receptor stimulation, CD27+ cells that concurrently were triggered via CD27 exhibited more resistance to apoptosis, interleukin 2 production, and proliferation than CD27- T cells. After transfer back into an HIV-infected patient, autologous HIV-specific CD27- T cells rapidly disappeared, but CD27+ T cells derived from the same clone persisted at high frequency. Our findings suggest that the CD27-CD70 interaction in HIV infection may provide CD27+ CD8+ T cells with a survival advantage and compensate for limiting or absent CD4+ T help to maintain the CD8 response.


http://www.jem.org/cgi/content/abstract/198/12/1841

Two important Ras guanine nucleotide exchange factors, Son of sevenless (Sos) and Ras guanine nucleotide releasing protein (RasGRP), have been implicated in controlling Ras activation when cell surface receptors are stimulated. To address the specificity or redundancy of these exchange factors, we have generated Sos1/Sos2 double- or RasGRP3-deficient B cell lines and determined their ability to mediate Ras activation upon B cell receptor (BCR) stimulation. The BCR requires RasGRP3; in contrast, epidermal growth factor receptor is dependent on Sos1 and Sos2. Furthermore, we show that BCR-induced recruitment of RasGRP3 to the membrane and the subsequent Ras activation are significantly attenuated in phospholipase C-(gamma)2-deficient B cells. This defective Ras activation is suppressed by the expression of RasGRP3 as a membrane-attached form, suggesting that phospholipase C-(gamma)2 regulates RasGRP3 localization and thereby Ras activation.


http://www.jem.org/cgi/content/abstract/196/12/1605

Apoptosis-associated speck-like protein containing a Caspase recruitment domain (ASC) belongs to a large family of proteins that contain a Pyrin, AIM, ASC, and death domain-like (PAAD) domain (also known as PYRIN, DAPIN, Pyk). Recent data have suggested that ASC functions as an adaptor protein linking various PAAD-family proteins to pathways involved in nuclear factor (NF)-{kappa}B and pro-Caspase-1 activation. We present evidence here that the role of ASC in modulating NF-{kappa}B activation pathways is much broader than previously suspected, as it can either inhibit or activate NF-{kappa}B, depending on cellular context. While coexpression of ASC with certain PAAD-family proteins such as Pyrin and Cryopyrin increases NF-{kappa}B activity, ASC has an inhibitory influence on NF-{kappa}B activation by various proinflammatory stimuli, including tumor necrosis factor (TNF){alpha}, interleukin 1{beta}, and lipopolysaccharide (LPS). Elevations in ASC protein levels or of the PAAD domain of ASC suppressed activation of I{kappa}B kinases in cells exposed to pro-inflammatory stimuli. Conversely, reducing endogenous levels of ASC using siRNA enhanced TNF- and LPS-induced degradation of the IKK substrate,
IκBα. Our findings suggest that ASC modulates diverse NF-κB induction pathways by acting upon the IKK complex, implying a broad role for this and similar proteins containing PAAD domains in regulation of inflammatory responses.


http://www.jem.org/cgi/content/abstract/198/1/161

A monolayer of pigment epithelium (PE) lines the iris PE (IPE), ciliary body PE, and retina PE of the inner eye, an immune-privileged site. These neural crest-derived epithelial cells participate in ocular immune privilege through poorly defined molecular mechanisms. Murine PE cells cultured from different ocular tissues suppress T cell activation by differing mechanisms. In particular, IPE cells suppress primarily via direct cell to cell contact. By examining surface expression of numerous candidate molecules (tumor necrosis factor receptor [TNFR]1, TNFR2, CD36, CD40, CD47, CD80, CD86, PD-L1, CD95 ligand, and type I interferon receptor), we report that IPE cells uniquely express on their surface the costimulatory molecule CD86. When IPE were blocked with anti-CD86 or were derived from CD80/CD86 (but not CD80) knockout (KO) mice, the cells displayed reduced capacity to suppress T cell activation. IPE also failed to suppress activation of T cells in the presence of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) immunoglobulin or if the T cells were obtained from CTLA-4 (but not CD28) KO mice. We conclude that iris pigment epithelial cells constitutively express cell surface CD86, which enables the cells to contact inhibit T cells via direct interaction with CTLA-4. Thus, ocular immune privilege is achieved in part by subversion of molecules that are usually used for conventional immune costimulation.


http://www.jem.org/cgi/content/abstract/201/5/779

Tumor environment can be critical for preventing the immunological destruction of antigenic tumors. We have observed a selective accumulation of CD4+CD25+ T cells inside tumors. In a murine fibrosarcoma Ld-expressing Ag104, these cells made up the majority of tumor-infiltrating lymphocytes at the late stage of tumor progression, and their depletion during the effector phase, rather than priming phase, successfully enhanced antitumor immunity. We show here that CD4+CD25+ T cells suppressed the proliferation and interferon-γ production of CD8+ T cells in vivo at the local tumor site. Blockade of the effects of IL-10 and TGF-β partially reversed the suppression imposed by the CD4+ cells. Furthermore, local depletion of CD4+ cells inside the tumor resulted in a change of cytokine milieu and led to the eradication of well-established highly aggressive tumors and the development of long-term antitumor memory. Therefore, CD4+CD25+ T cells maintained an environment in the tumor that concealed the immunogenicity of tumor cells to permit progressive growth of antigenic tumors. Our study illustrates that the suppression of antitumor immunity by regulatory T cells occurs predominantly at the tumor site, and that local reversal of suppression, even at a late stage of tumor development, can be an effective treatment for well-established cancers.

The low frequency of self-peptide-specific T cells in the human preimmune repertoire has so far precluded their direct evaluation. Here, we report an unexpected high frequency of T cells specific for the self-antigen Melan-A/MART-1 in CD8 single-positive thymocytes from human histocompatibility leukocyte antigen-A2 healthy individuals, which is maintained in the peripheral blood of newborns and adults. Postthymic replicative history of Melan-A/MART-1-specific CD8 T cells was independently assessed by quantifying T cell receptor excision circles and telomere length ex vivo. We provide direct evidence that the large T cell pool specific for the self-antigen Melan-A/MART-1 is mostly generated by thymic output of a high number of precursors. This represents the only known naive self-peptide-specific T cell repertoire directly accessible in humans.


The complete nucleotide sequence of Helicoverpa zea single-nucleocapsid nucleopolyhedrovirus (HzSNPV) has been determined (130869 bp) and compared to the nucleotide sequence of Helicoverpa armigera (Ha) SNPV. These two genomes are very similar in their nucleotide (97% identity) and amino acid (99% identity) sequences. The coding regions are much more conserved than the non-coding regions. In HzSNPV/HaSNPV, the 63 open reading frames (ORFs) present in all baculoviruses sequenced so far are much more conserved than other ORFs. HzSNPV has four additional small ORFs compared with HaSNPV, one of these (Hz42) being in a correct transcriptional context. The major differences between HzSNPV and HaSNPV are found in the sequence and organization of the homologous regions (hrs) and the baculovirus repeat ORFs (bro genes). The sequence identity between the HzSNPV and HaSNPV hrs ranges from 90% (hr1) to almost 100% (hr5) and the hrs differ in the presence/absence of one or more type A and/or B repeats. The three HzSNPV bro genes differ significantly from those in HaSNPV and may have been acquired independently in the ancestral past. The sequence data suggest strongly that HzSNPV and HaSNPV are variants of the same virus species, a conclusion that is supported by the physical and biological data.


Human parvovirus B19 (B19) infection during pregnancy is associated with the adverse foetal outcome known as non-immune hydrops fetalis (NIHF). Although B19 is known to infect erythroid-lineage cells in vivo as well as in vitro, the mechanism leading to the occurrence of NIHF is not
clear. To investigate the possible involvement of the B19 non-structural protein NS1 in NIHF, three independent lines of transgenic mice were generated that expressed NS1 under the control of the Cre-loxP system and the GATA1 promoter. Two of the three lines expressed NS1 in erythroid-lineage cells. Most of the transgenic mice died at the embryonic stage, some of which developed hydropic changes caused by severe anaemia at embryonic day 15{middle dot}5 (E15{middle dot}5). Histological examination of embryos at E15{middle dot}5 showed significantly fewer erythropoietic islands in the liver parenchyma, whereas their hearts showed no abnormal signs, such as cardiomegaly and apoptotic cells. The NS1-transgenic mouse lines established here provide an animal model for human NIHF and suggest that NS1 plays a crucial role in the adverse outcome associated with intrauterine B19 infection in humans.


http://vir.sgmjournals.org/cgi/content/abstract/83/4/855

Camelpox virus (CMPV) and variola virus (VAR) are orthopoxviruses (OPVs) that share several biological features and cause high mortality and morbidity in their single host species. The sequence of a virulent CMPV strain was determined; it is 202182 bp long, with inverted terminal repeats (ITRs) of 6045 bp and has 206 predicted open reading frames (ORFs). As for other poxviruses, the genes are tightly packed with little non-coding sequence. Most genes within 25 kb of each terminus are transcribed outwards towards the terminus, whereas genes within the centre of the genome are transcribed from either DNA strand. The central region of the genome contains genes that are highly conserved in other OPVs and 87 of these are conserved in all sequenced chordopoxviruses. In contrast, genes towards either terminus are more variable and encode proteins involved in host range, virulence or immunomodulation. In some cases, these are broken versions of genes found in other OPVs. The relationship of CMPV to other OPVs was analysed by comparisons of DNA and predicted protein sequences, repeats within the ITRs and arrangement of ORFs within the terminal regions. Each comparison gave the same conclusion: CMPV is the closest known virus to variola virus, the cause of smallpox.


http://vir.sgmjournals.org/cgi/content/abstract/86/5/1561

A new dsRNA was isolated from a Phytophthora isolate from Douglas fir. Sequence analysis showed the dsRNA to consist of 13 883 bp and to contain a single open reading frame with the potential to encode a polyprotein of 4548 aa. This polyprotein contained amino acid sequence motifs characteristic of virus RNA-dependent RNA polymerases (RdRps) in its C-terminal region and motifs characteristic of RNA helicases in its N-terminal region. These sequence motifs were related to corresponding motifs in plant viruses in the genus Endornavirus. In phylogenetic trees constructed from the RdRp and helicase motifs of a range of ssRNA and dsRNA viruses, the Phytophthora RdRp and helicase sequences clustered with those of the plant endornaviruses with good bootstrap support. The properties of the Phytophthora dsRNA are consistent with its being classified as the first non-plant member of the genus Endornavirus, for which we propose the name phytophthora endornavirus 1 (PEV1). A region between the RdRp and helicase domains of the PEV1 protein had significant amino acid sequence similarity to UDP glycosyltransferases (UGTs). Two sequence motifs were identified, one characteristic of all UGTs and the other characteristic of sterol UGTs. The PEV1 UGT would be the first for an RNA virus, although ecdysteroid UGT genes have been found in many baculoviruses. The PEV1 UGT was
only distantly related to baculovirus ecdysteroid UGTs, which belong to a family distinct from the sterol UGTs.


http://vir.sgmjournals.org/cgi/content/abstract/85/8/2327

The current dogma of influenza accepts that feral aquatic birds are the reservoir for influenza A viruses. Although the genomic information of human influenza A viruses is increasing, little of this type of data is available for viruses circulating in feral waterfowl. This study presents the genetic characterization of 35 viruses isolated from wild Canadian ducks from 1983 to 2000, as the first attempt at a comprehensive genotypic analysis of influenza viruses isolated from feral ducks. This study demonstrates that influenza virus genes circulating in Canadian ducks have achieved evolutionary stasis. The majority of these duck virus genes are clustered in distinct North American clades; however, some H6 and H9 genes are clustered with those from Eurasian viruses. Genes appeared to reassort in a random fashion. None of the genotypes identified remained present throughout all of the years examined and most PA and PB2 genes that crossed over into swine were clustered in one phylogenetic grouping. Additionally, matrix genes were identified that branch very early in the evolutionary tree. These findings demonstrate the diversity of the influenza virus gene pool in Canadian ducks, and suggest that genes which cluster in specific phylogenetic groupings in the PB2 and PA genes can be used for markers of viruses with the potential for crossing the species barrier. A more comprehensive study of this important reservoir is needed to provide further insight into the genomic composition of viruses that crossover the species barrier, which would be a useful component to pandemic planning.


http://vir.sgmjournals.org/cgi/content/abstract/85/2/283

A genomic characterization of hepatitis B virus (HBV) was done for 56 pre-S1/pre-S2 genes and 10 full-length HBV genotype C isolates from five Asian countries. Phylogenetic analysis of the pre-S1/pre-S2 genes revealed two major groups within genotype C: one for isolates from southeast Asia including Vietnam, Myanmar and Thailand (named HBV/C1) and the other for isolates from Far East Asia including Japan, Korea and China (named HBV/C2). This finding was confirmed by phylogenetic analysis based on the full-length sequence of 32 HBV genotype C isolates, including 22 from database entries. Two isolates from Okinawa, the island off the southern end of Japan, formed a different branch. Specific amino acid sequence changes were identified in the large S protein (amino acids 51, 54, 60, 62 and 73) and P protein (amino acids 231, 233, 236, 248, 252 and 304). Our results indicate that genotype C of HBV can be classified into at least two subgroups.


http://vir.sgmjournals.org/cgi/content/abstract/83/5/1049
Orf virus (ORFV) is the type species of the parapoxvirus genus and produces cutaneous pustular lesions in sheep, goats and humans. The genome encodes a polypeptide with remarkable homology to interleukin-10 (IL-10), particularly ovine IL-10, and also to IL-10-like proteins encoded by Epstein-Barr virus (EBV) and equine herpesvirus. IL-10 is a pleiotropic cytokine that can exert either immunostimulatory or immunosuppressive effects on many cell types. We have expressed and purified C-terminal FLAG and His6-tagged versions of ORFV-IL-10 and shown that ORFV-IL-10 costimulates murine mast cells (MC/9) and inhibits tumour necrosis factor-\(\alpha\) synthesis in activated mouse peritoneal macrophages. Our results demonstrate that although ORFV-IL-10 is structurally similar to EBV-IL-10 it has evolved a different spectrum of activities. EBV-IL-10 does not stimulate the proliferation of thymocytes or mast cells whereas ORFV-IL-10 has both of these activities. Recent studies show that the critical difference in molecular structure of human IL-10 and EBV-IL-10, which may be the basis of their functional differences, is linked to a single amino acid substitution. Consistent with the activity spectrum reported here for ORFV-IL-10, the viral gene encodes the critical amino acid seen in human IL-10. Although the ORFV-IL-10 gene has clearly undergone significant evolutionary change at the nucleotide level compared with ovine IL-10, it has largely retained the polypeptide structure and functional characteristics of its ovine counterpart, suggesting that mutations of the gene to a potentially more potent immunosuppressive form may compromise the co-existence of host and virus.


http://vir.sgmjournals.org/cgi/content/abstract/83/10/2497

To elucidate the structure of the antigenic sites of avian H5 influenza virus haemagglutinin (HA) we analysed escape mutants of a mouse-adapted variant of the H5N2 strain A/Mallard/Pennsylvania/10218/84. A panel of five anti-H5 monoclonal antibodies (mAbs) was used to select 16 escape mutants. The mutants were tested by ELISA and haemagglutination inhibition with this panel of anti-H5 mAbs and the HA genes of the mutants were sequenced. The sequencing demonstrated that the amino acid changes were grouped in two antigenic sites. One corresponded to site A in the H3 HA. The other contained areas that are separated in the amino acid sequence but are topographically close in the three-dimensional structure and partially overlap in the reactions with mAbs. This site corresponds in part to site B in the H3 structure; it also includes a region not involved in site B that partially overlaps site Sa in the H1 HA and an antigenic area in H2 HA. Mutants with the amino acid change K152N, as well as those with the change D126N, showed reduced lethality in mice. The substitution D126N, creating a new glycosylation site, was accompanied by an increase in the sensitivity of the mutants to normal mouse serum inhibitors. Several amino acid changes in the H5 escape mutants occurred at the positions of reported changes in H2 drift variants. This coincidence suggests that the antigenic sites described and analysed here may be important for drift variation if H5 influenza virus ever appears as a pathogen circulating in humans.


http://vir.sgmjournals.org/cgi/content/abstract/83/5/1189

Although muscovy duck reovirus (DRV) shares properties with the reovirus isolated from chicken, commonly named avian reovirus (ARV), the two virus species are antigenically different. Similar to the DRV \(\text{\sigma}B\)-encoded gene (1201 bp long) previously identified, the three other double-
stranded RNA small genome segments of DRV have been cloned and sequenced. They were 1325, 1191 and 1124 bp long, respectively, and contained conserved terminal sequences common to ARVs. They coded for single expression products, except the smallest (S4), which contained two overlapping open reading frames (ORF1 and ORF2). BLAST analyses revealed that the proteins encoded by the 1325 and 1191 bp genes shared high identity levels with ARV \{sigma\}A and \{sigma\}NS, respectively, and to a lesser extent with other orthoreovirus counterparts. No homology was found for the S4 ORF1-encoded p10 protein. The 29\(\text{dot}4\) kDa product encoded by S4 ORF2 appeared to be 25\% identical to ARV S1 ORF3-encoded \{sigma\}C, a cell-attachment oligomer inducing type-specific neutralizing antibodies. Introduction of large gaps in the N-terminal part of the DRV protein was necessary to improve DRV and ARV \{sigma\}C amino acid sequence alignments. However, a leucine zipper motif was conserved and secondary structure analyses predicted a three-stranded \{alpha\}-helical coiled-coil feature at this amino portion. Thus, despite extensive sequence divergence, DRV \{sigma\}C was suggested to be structurally and probably functionally related to ARV \{sigma\}C. This work provides evidence for the diversity of the polycistronic S class genes of reoviruses isolated from birds and raises the question of the relative classification of DRV in the Orthoreovirus genus.


http://vir.sgmjournals.org/cgi/content/abstract/86/4/1121

The severity of disease caused in humans by H5N1 influenza viruses remains unexplained. The NS gene of Hong Kong H5N1/97 viruses was shown to contribute to high pathogenicity of reassortants in a pig model. However, the molecular pathogenesis and host immune response underlying this phenomenon remain unclear. Here, in a mouse model, H1N1 A/Puerto Rico/8/34 (PR/8) reassortants that contained the H5N1/97 NS gene, the H5N1/01 NS gene, or an altered H5N1/97 NS gene encoding a Glu92\(-\rightarrow\)Asp substitution in NS1 was studied. The pathogenicity of reassortant viruses, the induction of cytokines and chemokine CXCL1 (KC) in the lungs and specific B- and T-cell responses was characterized. In mice infected with reassortant virus containing the H5N1/97 NS gene, the mouse lethal dose (50 \%) and lung virus titres were similar to those of PR/8, which is highly pathogenic to mice. This reassortant virus required two more days than PR/8 to be cleared from the lungs of infected mice. Reassortants containing the altered H5N1/97 NS gene or the H5N1/01 NS gene demonstrated attenuated pathogenicity and lower lung titres in mice. Specific B- and T-cell responses were consistent with viral pathogenicity and did not explain the delayed clearance of the H5N1/97 NS reassortant. The reassortant induced elevated pulmonary concentrations of the inflammatory cytokines IL1\{alpha\}, IL1\{beta\}, IL6, IFN-\{gamma\} and chemokine KC, and decreased concentrations of the anti-inflammatory cytokine IL10. This cytokine imbalance is reminiscent of the clinical findings in two humans who died of H5N1/97 infection and may explain the unusual severity of the disease.


http://vir.sgmjournals.org/cgi/content/abstract/85/2/441

Variants of hepatitis C virus (HCV) from a single infected blood donor and 13 viraemic recipients who were traced were examined by sequencing and cloning to determine the extent of virus diversity in hypervariable region 1. Serum-derived viral isolates were studied from the donor when his HCV infection was discovered in 1993, in his recipients that year (0\(\text{dot}3\)-5 years post-
transfusion) and 5 years later in the donor and six viraemic recipients who were still alive. Viral variants of broad diversity were readily demonstrated in the baseline samples of the donor (nucleotide p-distance 0.130), but significantly less (P<0.00003) diversity was observed in the recipients' first samples (p-distances within recipients 0.003-0.062). In the first blood samples of the recipients, many of the viral variants identified were closely related to a strain variant from the donor. In follow-up samples drawn 5 years later from the donor and six recipients, the p-distance among donor clones had increased (0.172, P<0.0005) compared with the recipients, who displayed significantly narrower quasispecies (0.011-0.086). A common finding was that recipients of blood components processed from the same donation differed substantially in persisting HCV infectious sequence. Markedly few changes leading to changes of amino acids had occurred during follow-up in four of six recipients. These results question the significance of the development of viral variants as a necessary phenomenon in the evolution of HCV and pathogenesis of the disease.


http://vir.sgmjournals.org/cgi/content/abstract/85/1/137

A regulated switch between latent and lytic gene expression is common to all known herpesviruses. However, the effects on host colonization of altering this switch are largely unknown. We deregulated the transcription of the gene encoding the major lytic transactivator of murine gammaherpesvirus-68, ORF50, by inserting a new and powerful promoter element in its 5' untranslated region. In vitro, the mutant virus (M50) transcribed ORF50 at a high level and showed more rapid lytic spread in permissive fibroblast cultures, but in vivo, the M50 virus showed a severe deficit in latency establishment, with no sign of the infectious mononucleosis-like illness normally associated with wild-type infection. Although a low level of M50 viral DNA was detectable by PCR in spleens, replication-competent virus could not be recovered beyond 10 days post-infection. The M50 virus was also attenuated in immunocompromised mice. Thus a gammaherpesvirus unable to shut off lytic cycle gene expression showed severely restricted host colonization.


http://vir.sgmjournals.org/cgi/content/abstract/84/11/3087

Pre-weaning diarrhoea is a well-known problem in mink farming in Europe, causing morbidity that varies between farms, regions and season. Different causalities for the disease have been proposed, but only most recently has a novel astrovirus been identified as an important risk factor. In this report, the molecular characterization, origin and evolution of this novel astrovirus of mink are discussed. The polyadenylated, positive-stranded RNA genome was sequenced and found to contain 6610 nt, organized into three ORFs and two short UTRs. A ribosomal frameshift sequence links the 5' two ORFs, containing sequence motifs for a serine protease (ORF1a) and an RNA-dependent RNA polymerase (ORF1b). The structural proteins are encoded by ORF2 and, presumably, are expressed as a polyprotein precursor to be cleaved into the mature capsid proteins. These results indicate that mink astrovirus (MiAstV) has all of the features typical of members of the Astroviridae. Phylogenetic analyses revealed that MiAstV is distantly related to established astroviruses, showing less than 67 % similarity at the nucleotide level with its closest relative, ovine astrovirus, and even lower identities at the predicted amino acid level. Nevertheless, sequence analysis of MiAstV isolates from geographically distinct Swedish and Danish farms showed much less diversity. This suggests either the spread in the mink population
of a virus that has evolved a long time ago or the recent introduction of an ancient virus into a new host species.


http://vir.sgmjournals.org/cgi/content/abstract/85/2/495

The initial characterization of a rhabdovirus isolated from a single, asymptomatic starry flounder (Platichthys stellatus) collected during a viral survey of marine fishes from the northern portion of Puget Sound, Washington, USA, is reported. Virions were bullet-shaped and approximately 100 nm long and 50 nm wide, contained a lipid envelope, remained stable for at least 14 days at temperatures ranging from -80 to 5 (degrees)C and grew optimally at 15 (degrees)C in cultures of epithelioma papulosum cyprini (EPC) cells. The cytopathic effect on EPC cell monolayers was characterized by raised foci containing rounded masses of cells. Pyknotic and dark-staining nuclei that also showed signs of karyorrhexis were observed following haematoxylin and eosin, May-Grunwald Giemsa and acridine orange staining. PAGE of the structural proteins and PCR assays using primers specific for other known fish rhabdoviruses, including Infectious hematopoietic necrosis virus, Viral hemorrhagic septicemia virus, Spring viremia of carp virus, and Hirame rhabdovirus, indicated that the new virus, tentatively termed starry flounder rhabdovirus (SFRV), was previously undescribed in marine fishes from this region. In addition, sequence analysis of 2678 nt of the amino portion of the viral polymerase gene indicated that SFRV was genetically distinct from other members of the family Rhabdoviridae for which sequence data are available. Detection of this virus during a limited viral survey of wild fishes emphasizes the void of knowledge regarding the diversity of viruses that naturally infect marine fish species in the North Pacific Ocean.


http://vir.sgmjournals.org/cgi/content/abstract/85/5/1191

We investigated the protective efficacy of a systemic triple vector (DNA/rSFV/rMVA)-based vaccine against mucosal challenge with pathogenic simian immunodeficiency virus (SIV) in cynomolgus monkeys. Animals were immunized at week 0 with DNA (intradermally), at weeks 8 and 16 with recombinant Semliki Forest virus (rSFV, subcutaneously) and finally, at week 24, with recombinant modified vaccinia virus Ankara strain (rMVA, intramuscularly). Both DNA and recombinant viral vectors expressed a wide range of SIV proteins (Gag, Pol, Tat, Rev, Env and Nef). This immunization strategy elicited cell-mediated rather than humoral responses that were especially increased following the last boost. Upon intrarectal challenge with pathogenic SIVmac251, three of the four vaccinated monkeys dramatically abrogated virus load to undetectable levels up to 41 weeks after challenge. A major contribution to this vaccine effect appeared to be the T-cell-mediated immune response to vaccine antigens (Gag, Rev, Tat, Nef) seen in the early phase of infection in three of the four vaccinated monkeys. Indeed, the frequency of T-cells producing antigen-induced IFN-gamma mirrored virus clearance in the vaccinated and protected monkeys. These results, reminiscent of the efficacy of live attenuated virus vaccines, suggest that vaccination with a combination of many viral antigens can induce a robust and stable vaccine-induced immunity able to abrogate virus replication.
Hepatitis C virus (HCV) is a major cause of liver disease. Knowledge of HCV variability is crucial for clinical and epidemiological analysis. HCV genotype 4 (HCV-4) has become increasingly prevalent in European countries, including France, in recent years. The present study investigates the heterogeneity of HCV-4 in south-western France by phylogenetic analysis of NS5B sequences from 166 patients. The E2 region of 90 strains was also analysed. Genotype 4 accounts for 7.4% of HCV infections in this area. Analysis of the NS5B region revealed 12 subtypes and the NS5B and E2 phylogeny data were congruent, except for one strain. The epidemiological data indicated two main groups of patients. One included intravenous drug users (IVDUs) of French origin, who were infected by homogeneous strains of subtypes 4a or 4d. The second group comprised non-IVDU patients who were infected with heterogeneous strains. This group was subdivided into patients of French origin, who were infected with eight subtypes, and patients from non-European countries (Central Africa or the Middle East), who were mainly infected with 4f, 4k, 4r and other subtypes; they showed the greatest genetic heterogeneity. This study of a large cohort of patients shows the great diversity of HCV-4 strains, and that these subtypes have spread differently.

The complete genome sequence of a new isolate of enzootic nasal tumour virus (ENTV-2), associated with enzootic nasal adenocarcinoma (ENA) of goats, was determined. The genome exhibits a genetic organization characteristic of {beta}-retroviruses. ENTV-2 is closely related to the retrovirus (ENTV-1) associated with enzootic adenocarcinoma of sheep, and to jaagsiekte retrovirus. The main sequence differences between these viruses reside in orfX, the U3 LTR, two small regions in gag and the transmembrane (TM) region of env. Sequence analysis of the TM region of env from several sheep and goats naturally affected by ENA suggested that ENTV-1 and ENTV-2 are distinct viruses rather than geographical variants. Although both viruses transform secretory epithelial cells of the ethmoid turbinate, the study of their tissue distribution using specific PCRs showed that ENTV-2 establishes a disseminated lymphoid infection whereas ENTV-1 is mainly confined to the tumour.

Human papillomaviruses (HPVs) are aetiological agents of human malignancies, most notably cervical cancers. The life-cycles of HPVs are dependent on epithelial differentiation, and this has impeded many basic studies of HPV biology. The organotypic (raft) culture system supports epithelial differentiation such that infectious virions are synthesized in raft tissues from epithelial cells that replicate extrachromosomal HPV genomes. The CIN-612 9E cell line maintains episomal copies of HPV type 31b (HPV31b), an HPV type associated with cervical cancers. Many
previous studies, including our own, have focused on characterizing the later stages of the HPV31b life-cycle in CIN-612 9E raft tissues. In this study, we have used the raft system to generate large numbers of HPV31b viral DNA (vDNA)-containing particles. We found a biologically contained homogenization system to be efficient at virion extraction from raft epithelial tissues. We also determined that vDNA-containing particles could be directly quantified from density-gradient fractions. Using an RT-PCR assay, the presence of newly synthesized, spliced HPV31b transcripts was detected following HPV31b infection of the immortalized HaCaT epithelial cell line. Spliced E6 and E1(\wedge)E4 RNAs were detected using a single round of RT-PCR from cells infected with a dose as low as 1 vDNA-containing particle per cell. Spliced E1*E2 transcripts were found in cells infected with an HPV31b dose as low as 10 vDNA-containing particles per cell. Infectivity was blocked by HPV31 antiserum, but was not affected by DNase I. This work lays a foundation for a detailed analysis of the early events in HPV infection.


http://vir.sgmjournals.org/cgi/content/abstract/84/3/647

Human cytomegalovirus (HCMV) ORF UL73 is a polymorphic locus, encoding the viral glycoprotein gpUL73-gN, a component of the gC-II envelope complex. The previously identified gN genomic variants, denoted gN-1, gN-2, gN-3 and gN-4, were further investigated in this work by analysing a large panel of HCMV clinical isolates collected from all over the world (223 samples). Sequencing and phylogenetic analysis confirmed the existence of the four gN genotypes, but also allowed the identification of a novel subgroup belonging to the gN-3 genotype, which was designated gN-3b. The number of non-synonymous (dN) and synonymous (dS) nucleotide substitutions and their ratio (dN/dS) were estimated among the gN genotypes to evaluate the possibility of positive selection. Results showed that the four variants evolved by neutral (random) selection, but that the gN-3 and gN-4 genotypes are maintained by positive selective pressure. The 223 HCMV clinical isolates were subdivided according to their geographical origin, and four main regions of gN prevalence were identified: Europe, China, Australia and Northern America. The gN variants were found to be widespread and represented within the regions analysed without any significant difference, and no new genotype was detected. Finally, for clinical and epidemiological purposes, a rapid and low-cost method for genetic grouping of the HCMV clinical isolates was developed based on the RFLP revealed by SacI, Scal and Sall digestion of the PCR-amplified UL73 sequence. This technique enabled us to distinguish all four gN genomic variants and also their subtypes.


http://vir.sgmjournals.org/cgi/content/abstract/86/3/773

Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neurological disease observed only in 1-2 % of infected individuals. HTLV-1 provirus load, certain HLA alleles and HTLV-1 tax subgroups are reported to be associated with different levels of risk for HAM/TSP in Kagoshima, Japan. Here, it was determined whether these risk factors were also valid for HTLV-1-infected individuals in Mashhad in northeastern Iran, another region of endemic HTLV-1 infection. In Iranian HTLV-1-infected individuals (n=132, 58 HAM/TSP patients and 74 seropositive asymptomatic carriers), although...
HLA-DRB1*0101 was associated with disease susceptibility in the absence of HLA-A*02 (P=0.038; odds ratio=2.71) as observed in Kagoshima, HLA-A*02 and HLA-Cw*08 had no effect on either the risk of developing HAM/TSP or HTLV-1 provirus load. All Iranian subjects possessed tax subgroup A sequences, and the protective effects of HLA-A*02 were observed only in Kagoshima subjects with tax subgroup B but not in those with tax subgroup A. Both the prevalence of HTLV-1 subgroups and the host genetic background may explain the different risks levels for HAM/TSP development in these two populations.


http://vir.sgmjournals.org/cgi/content/abstract/85/8/2421

Porcine endogenous retroviruses (PERV) are of concern when the microbiological safety aspects of xenotransplantation are considered. Four unique isolates of PERV B have been identified previously from a lambda library constructed from genomic DNA from a Large White pig. This study shows that none of these isolates are replication competent when transfected into permissive human or pig cells in vitro, and the removal of flanking genomic sequences does not confer a human tropic replication competent (HTRC) phenotype on these PERV proviruses. Analysis of the envelope sequences revealed that PERV B demonstrated high similarity to the envelope sequences derived from replication-competent PERV, indicating that lack of replication competence does not appear to be attributable to this region of the provirus. These data complement recent findings that HTRC PERV are recombinants between the PERV A and PERV C subgroups, and that these recombinants are not present in the germline of miniature swine. Together, these results indicate that these individual PERV B proviruses are unlikely to give rise to HTRC PERV.


http://vir.sgmjournals.org/cgi/content/abstract/84/8/2009

Malignant catarrhal fever (MCF) is a herpesvirus disease syndrome of ruminants. The microscopic pathology of MCF is characterized by lymphoid proliferation and infiltration, necrotizing vasculitis and epithelial necrosis. Because previous attempts to detect viral antigen or nucleic acids in lesions have been unsuccessful, the pathogenesis of the lesions in acute MCF has been speculated to involve mechanisms of autoimmunity and lymphocyte dysregulation. In this study, the vascular lesions in the brains of a cow and a bison with acute MCF were evaluated by in situ PCR and immunohistochemistry. The results demonstrated that the predominant infiltrating cell type in these lesions was CD8+ T lymphocytes and that large numbers of these cells were infected with ovine herpesvirus 2. The lesions also contained macrophages, but no detectable CD4+ or B lymphocytes.

Avian hepatitis E virus (HEV), a novel virus identified from chickens with hepatitis-splenomegaly (HS) syndrome, is genetically and antigenically related to human HEV. Recently, it was found that avian HEV antibody is also prevalent in healthy chickens. A prospective study was done on a known seropositive but healthy chicken farm to identify avian HEV isolates from healthy chickens. Fourteen chickens were randomly selected, tagged and monitored under natural conditions for 19 weeks. All 14 chickens were seronegative at the beginning of the study at 12 weeks of age. By 21 weeks of age, all 14 chickens had seroconverted to avian HEV antibody. None of the chickens had any sign of HS syndrome. Partial helicase gene and capsid gene sequences of avian HEV isolates recovered from a healthy chicken were determined and found to share 75-97 % nucleotide sequence identity with the corresponding regions of avian HEV isolates from chickens with HS syndrome. Thus far, only one strain of avian HEV from a chicken with HS syndrome has been genetically characterized for its capsid gene, therefore the capsid gene region of an additional 14 isolates from chickens with HS syndrome were also characterized. The capsid genes of avian HEV isolates from chickens with HS syndrome were found to be heterogeneous, sharing 76-100 % nucleotide sequence identity with each other. This study indicates that avian HEV is enzootic in chicken flocks and spreads subclinically among chickens in the United States and that the virus is heterogeneous.


The pathogenesis of strain 3711 of the chicken anemia virus (CAV), propagated in chickens, and two preparations of strain 3711 that had been adapted to grow to high titre in cells of the MDCC-MSB1 line were studied in chicken embryos and/or chickens. Highest viral loads in infected chickens, as measured by a microplate DNA-hybridization assay, were detected in the thymus, clotted blood and pancreas, and the lowest in the duodenum. The CAV DNA copy number in the organs of chicken embryos was significantly lower than in chickens. Route of infection was an important determinant of the course of disease in chickens, with clinical signs appearing earlier in birds infected by the intramuscular than those infected by the oral route; there was a direct relationship between viral load in particular organs and the extent of clinical signs. No reduction in the pathogenicity for chickens was noted for strain 3711 after 65 or 129 passages in the MDCC-MSB1 cell line.


So-called fowl glioma is a retroviral infectious disease caused by avian leukosis virus subgroup A (ALV-A). We determined the complete nucleotide sequence of the virus genome. The full-length sequence was consistent with a genetic organization typical of a replication-competent type C retrovirus lacking viral oncogenes. The coding sequences were well conserved with those of replication-competent viruses, but the 3' noncoding regions including LTR were most related to those of replication-defective sarcoma viruses. The U3 region of the LTR had a few deletions and several point mutations compared to that of other ALVs. The promoter activities of the LTRs of glioma-inducing ALV and ALV-A standard strain, RAV-1, were equivalent in chick embryo
fibroblasts (CEF), while that of glioma-inducing ALV was significantly lower than that of RAV-1 in human astrocytic cells. These subtle differences of the promoter activity of the LTR may be related to the induction of glial neoplasm.


http://vir.sgmjournals.org/cgi/content/abstract/83/11/2869

The complete nucleotide sequence of an ophiovirus associated with lettuce big-vein disease has been elucidated. The genome consisted of four RNA molecules of approximately 7·8, 1·7, 1·5 and 1·4 kb. Virus particles were shown to contain nearly equimolar amounts of RNA molecules of both polarities. The 5' and 3'-terminal ends of the RNA molecules are largely, but not perfectly, complementary to each other. The virus genome contains seven open reading frames. Database searches with the putative viral products revealed homologies with the RNA-dependent RNA polymerases of rhabdoviruses and Ranunculus white mottle virus, and the capsid protein of Citrus psorosis virus. The gene encoding the viral polymerase appears to be located on the RNA segment 1, while the nucleocapsid protein is encoded by the RNA3. No significant sequence similarities were observed with other viral proteins. In spite of the morphological resemblance with species in the genus Tenuivirus, the ophioviruses appear not to be evolutionary closely related to this genus nor any other viral genus.


http://vir.sgmjournals.org/cgi/content/abstract/83/12/3131

The complete nucleotide sequence of the genomic RNA of an aphid-infecting virus, Aphid lethal paralysis virus (ALPV), has been determined. The genome is 9812 nt in length and contains two long open reading frames (ORFs), which are separated by an intergenic region of 163 nt. The first ORF (5' ORF) is preceded by an untranslated leader sequence of 506 nt, while an untranslated region of 571 nt follows the second ORF (3' ORF). The deduced amino acid sequences of the 5' ORF and 3' ORF products respectively showed similarity to the non-structural and structural proteins of members of the newly recognized genus Cripavirus (family Dicistroviridae). On the basis of the observed sequence similarities and identical genome organization, it is proposed that ALPV belongs to this genus. Phylogenetic analysis showed that ALPV is most closely related to Rhopalosiphum padi virus, and groups in a cluster with Drosophila C virus and Cricket paralysis virus, while the other members of this genus are more distantly related. Infectivity experiments showed that ALPV can not only infect aphid species but is also able to infect the whitefly Trialeurodes vaporariorum, extending its host range to another family of the order Hemiptera.


http://vir.sgmjournals.org/cgi/content/abstract/84/1/165

The genomic sequence of a new icosahedral DNA virus infecting Myzus persicae has been determined. Analysis of 5499 nt of the viral genome revealed five open reading frames (ORFs)
evenly distributed in the 5' half of both DNA strands. Three ORFs (ORF1-3) share the same strand, while two other ORFs (ORF4 and ORF5) are detected in the complementary sequence. The overall genomic organization is similar to that of species from the genus Densovirus. ORFs 1-3 most likely encode the non-structural proteins, since their putative products contain conserved replication motifs, NTP-binding domains and helicase domains similar to those found in the NS-1 protein of paroviruses. The deduced amino acid sequences from ORFs 4 and 5 show sequence similarities with the structural proteins of the members of the genus Densovirus. These data indicate that this virus is a new species of the genus Densovirus in the family Paroviridae. The virus was tentatively named Myzus persicae densovirus.


http://biomed.gerontologyjournals.org/cgi/content/abstract/60/3/285

Age-related alterations of DNA repair could be involved in the accumulation of genetic damage with age. Few data suggest a possible alteration with age of the mismatch repair system, evidenced by the acquisition of microsatellite instability. We aimed to point out a possible implication of this repair system in the accumulation of genetic damage with age. Peripheral blood cell DNA from 226 participants, 110 young (25-35 years), 58 old (85-97 years), and 58 centenarian was analyzed at five polymorphic microsatellite loci (CD4, p53, VWA31, TPOX, and FES/FPS) to point out age-related instabilities or modifications in allele frequencies. FES/FPS microsatellite was the most instable, showing both the appearance of trizygosis in DNA from old participants and differences in allele patterns among age groups, thus indicating an association between increased microsatellite instability and aging, one of the possible causes of which being an impairment of mismatch repair system capacity with age.

J. Hered. (16)


http://jhered.oupjournals.org/cgi/content/abstract/esi036v2

Giant squids (Architeuthis sp.) remain mysterious; they have evaded observation and are rarely taken from their deep sea habitat. Information on the diet of Architeuthis is scarce due to the limited number of specimens with morphologically recognizable remains in their digestive tracts. We explored the use of polymerase chain reaction (PCR)-based methods for detection of DNA in the prey remains and amorphous slurry from an Architeuthis gut sample. The DNA region amplified varied in size, allowing separation of fish and squid components. Sequence
comparisons identified fish prey as Macruronus novaezelandiae. Isolation of Architeuthis DNA from an ingested tentacle and the presence of chitin fragments indicate cannibalism occurs in giant squid. Denaturing gradient gel electrophoresis was used to screen for less common DNA types, revealing a high frequency of PCR-generated false alleles, but no additional prey species.


The Chondrostoma genus is widespread in Europe, with numerous endemic species in northern Mediterranean rivers. We reconstructed the phylogenetic relationships of this genus, using the whole cytochrome b sequence and compared the two freshwater fish dispersion hypotheses: (1) dispersion around the Mediterranean Sea during the Lago Mare phase of the Messinian salinity crisis (Bianco's hypothesis) and (2) an older and more gradual colonization of the Mediterranean rivers (Banarescu's hypothesis). All phylogenetic analyses identified two levels of divergences, implying two radiation events in the Chondrostoma genus. The first radiation mainly concerned Mediterranean species, whereas the second one includes Danubian and Mesopotamian species. This phylogeographic pattern was already observed for the genus Squalius, which exhibits a similar geographic range distribution in Europe and probably is shared with several other Mediterranean genera, such as Scardinius, Rutilus, and Telestes. Furthermore, assuming a molecular clock of 1% per million years, the first radiation appears consistent with a Messinian dispersion during the Lago Mare, 5.3 million years ago, whereas the second one may correspond to a Mesopotamian dispersion through the Black Sea to the Danube system. According to our results, the Lago Mare theory is strengthened, and a more recent and pre-Pleistocene colonization of the Danube from Mesopotamian freshwater fishes is suggested.


Mutations in Cu/Zn superoxide dismutase (SOD1), a major cytosolic antioxidant enzyme in eukaryotic cells, have been reported in approximately 20% of familial amyotrophic lateral sclerosis (FALS) patients. Hereditary canine spinal muscular atrophy (HCSMA), a fatal inherited motor neuron disease in Brittany spaniels, shares many clinical and pathological features with human motor neuron disease, including FALS. The SOD1 coding region has been sequenced and cloned from several animal species, but not from the dog. We have mapped the chromosomal location, sequenced, and characterized the canine SOD1 gene. Extending this analysis, we have evaluated SOD1 as a candidate for HCSMA. The 462 bp SOD1 coding region in the dog encodes 153 amino acid residues and exhibits more than 83% and 79% sequence identity to other mammalian homologues at both the nucleotide and amino acid levels, respectively. The canine SOD1 gene maps to CFA31 close to syntenic group 13 on the radiation hybrid (RH) map in the vicinity of sodium myo/inositol transporter (SMIT) gene. The human orthologous SOD1 and SMIT genes have been localized on HSA 21q22.1 and HSA 21q21, respectively, confirming the conservation of synteny between dog syntenic group 13 and HSA 21. Direct sequencing of SOD1 cDNA from six dogs with HCSMA revealed no mutations. Northern analysis indicated no differences in steady-state levels of SOD1 mRNA.
In this article we present the first analysis of parentage and relatedness in a natural vertebrate population, using Intersimple Sequence Repeat (ISSR) markers. Thus, 28 ISSR markers were used in a study of a sex-role reversed, simultaneously polyandrous shorebird from northeastern Australia, the comb-crested jacana (Irediparra gallinacea). Assessment of parentage was based on comparison of field observations, novel bands, individual-specific bands found in 7/9 males and 4/6 females, and a 99% CI exclusion criteria. Integrating results from these approaches resulted in confirmation of paternity in all 36 chicks. In only one case (2.8% of chicks) was a co-mate assigned paternity. Thus, comb-crested jacanas appear to be genetically monogamous. These results showed resemblance to sequentially polyandrous birds but differed from the simultaneously polyandrous wattled jacana (Jacana jacana; Emlen et al. 1998). A significant relationship between relatedness and ISSR similarity resulted in recognition that 14/15 adults sampled may be related to at least one other adult by 0.25 or more. Lack of dispersal may be explained by physical limitations and adequate regional habitat. ISSRs proved to be simple and helpful in resolving these issues.

The emu (Dromaius novaehollandiae) occupies most regions of the Australian continent and in recent times has been farmed for meat, oil, and leather. Very little is known about the genetic structure of natural or farmed populations of these birds. We report a preliminary study of genetic variation in emus undertaken by typing birds from five farms and two natural populations at five polymorphic microsatellite loci. Genetic diversity was high for all populations and there was little evidence of inbreeding, with most populations conforming to Hardy-Weinberg equilibrium for most loci. Significant heterozygote deficiencies at one locus in a number of populations were detected and may indicate the presence of null alleles. Comparisons of allele frequencies showed little evidence of genetic differentiation either among farmed populations or between farmed and natural populations.

The Sox9 gene of Acipenser sturio, one of the most primitive vertebrates, was analyzed. No sex-specific differences were observed. Sturgeon Sox9 consists of three exons and two introns with completely conserved exon-intron boundaries showing high levels of homology to other vertebrate Sox9 sequences, especially in the N-terminus region containing the HMG box. We found strong evidence for negative (purifying) selection. In contrast to previous studies of other fishes, we observed no evidence for gene duplication in sturgeon. Phylogenetic analyses of Sox9 evolution revealed a basal position for sturgeon Sox9.
Dog breeds were created by man choosing for select phenotypic traits such as size, shape, coat color, conformation, and behavior. Rigorous phenotypic selection likely resulted in a loss of genetic information. The present study extends previous dog population observations by assessing the genotypic variation within and across 28 breeds representing the seven recognized breed groups of the American Kennel Club (AKC). One hundred autosomal microsatellite markers distributed across the canine genome were used to examine variation within breeds. Resulting breed-specific allele frequencies were then used in an attempt to elucidate phylogeny and genetic distances between breeds. While the set of autosomal microsatellites was useful in describing genetic variation within breeds, establishing the genetic relatedness between breeds was less conclusive. A more accurate determination of breed phylogeny will likely require the use of single-nucleotide polymorphisms (SNPs).


In this study we determined the complete sequence of the mitochondrial DNA (mtDNA) control region of the Eurasian otter (Lutra lutra). We then compared these new sequences with orthologues of nine carnivores belonging to six families (Mustelidae, Mephitidae, Canidae, Hyaenidae, Ursidae, and Felidae). The comparative analyses identified all the conserved regions previously found in mammals. The Eurasian otter and seven other species have a single location with tandem repeats in the right domain, while the spotted hyena (Hyaenidae) and the tiger (Felidae) have repeated sequences in both the right and left domains. To assess the degree of genetic heterogeneity of the Eurasian otter in Italy we sequenced two fragments of the gene and analyzed length polymorphisms of repeated sequences and heteroplasmy in 32 specimens. The study includes 23 museum specimens collected in northern, central, and southern Italy; most of these specimens are from extinct populations, while the southern Italian samples belong to the sole extant Italian population of the Eurasian otter. The study also includes all the captive-reared animals living in the colony "Centro Lontra, Caramanico Terme" (Pescara, central Italy). The colony is maintained for reintroduction of the species. We found a low level of genetic polymorphism; a single haplotype is dominant, but our data indicate the presence in central and southern Italy of two slightly divergent haplotypes. One haplotype belongs to an extinct population, the other is present in the single extant Italian population. Analyses of length polymorphisms and heteroplasmy indicate that the autochthonous Italian samples are characterized by a distinct array of repeated sequences from captive-reared animals.


A complete DNA-based inventory of the Earth's present biota using large-scale high-throughput DNA sequencing of signature region(s) (DNA barcoding) is an ambitious proposal rivaling the Human Genome Project. We examine whether this approach will also enable us to assess the
past diversity of the earth’s biota. To test this, we sequenced the 5’ terminus of the mitochondrial cytochrome c oxidase I (COI) gene of individuals belonging to a group of extinct ratite birds, the moa of New Zealand. Moa comprised a large number of taxa that radiated in isolation on this oceanic landmass. Using a phylogenetic approach based on a large data set including protein coding and 12S DNA sequences as well as morphology, we now have precise information about the number of moa species that once existed. We show that each of the moa species detected using this extensive data set has a unique COI barcode(s) and that they all show low levels of within-species COI variation. Consequently, we conclude that COI sequences accurately identify the species discovered using the larger data set. Hence, more generally, this study suggests that DNA barcoding might also help us detect other extinct animal species and that a large-scale inventory of ancient life is possible.


http://jhered.oupjournals.org/cgi/content/abstract/94/1/95

We report construction of second-generation integrated genetic linkage and radiation hybrid (RH) maps in the domestic cat (Felis catus) that exhibit a high level of marker concordance and provide near-full genome coverage. A total of 864 markers, including 585 coding loci (type I markers) and 279 polymorphic microsatellite loci (type II markers), are now mapped in the cat genome. We generated the genetic linkage map utilizing a multigeneration interspecies backcross pedigree between the domestic cat and the Asian leopard cat (Prionailurus bengalensis). Eighty-one type I markers were integrated with 247 type II markers from a first-generation map to generate a map of 328 loci (320 autosomal and 8 X-linked) distributed in 47 linkage groups, with an average intermarker spacing of 8 cM. Genome coverage spans approximately 2,650 cM, allowing an estimate for the genetic length of the sex-averaged map as 3,300 cM. The 834-locus second-generation domestic cat RH map was generated from the incorporation of 579 type I and 255 type II loci. Type I markers were added using targeted selection to cover either genomic regions underrepresented in the first-generation map or to refine breakpoints in human/feline synteny. The integrated linkage and RH maps reveal approximately 110 conserved segments ordered between the human and feline genomes, and provide extensive anchored reference marker homologues that connect to the more gene dense human and mouse sequence maps, suitable for positional cloning applications.


http://jhered.oupjournals.org/cgi/content/abstract/95/2/158

Y chromosome polymorphisms such as microsatellites or single nucleotide polymorphisms represent a paternal counterpart to mitochondrial DNA (mtDNA) for evolutionary and phylogeographic studies. The use of Y chromosome haplotyping in natural populations of species other than humans is still hindered by the lack of sequence information necessary for polymorphism screening. Here we used representational difference analysis (RDA) followed by a screen of a bacterial artificial chromosome (BAC) library for repetitive sequences to obtain polymorphic Y-chromosomal markers. The procedure was performed for the domestic horse (Equus caballus) and we report the first six Y-chromosomal microsatellite markers for this species. Three markers were also useful for haplotyping taxa of the zebra/ass lineage. Y-chromosomal microsatellite markers show a single haplotype in the domestic horse, whereas notable variation has been observed in the other members of the genus Equus.

http://jhered.oupjournals.org/cgi/content/abstract/95/2/144

Population bottlenecks may lead to diminished genetic variability and correlative effects on fitness. The Guadalupe fur seal was nearly exterminated by commercial sealers during the late 18th and early 19th centuries. To determine the genetic consequences of this population bottleneck, we compared the variation at a 181 bp section of the mitochondrial DNA (mtDNA) control region from the bones of 26 prebottleneck fur seals versus variation in the extant population. We found 25 different mtDNA genotypes in the prebottleneck fur seals and only 7 genotypes among 32 extant fur seals, including only one of the ancient genotypes. These data demonstrate a substantial loss of genetic variability correlating with the recent population bottleneck. We also found from several genetic measures that the prehistoric population of Guadalupe fur seals was robust and that it had been increasing at some time during the late prehistoric period. Continued recovery of this species may, however, owe more to more immediate nongenetic factors, such as poaching and local availability of food resources during the breeding season and consequent effects on pup survival, than on the reduced genetic variability.


http://jhered.oupjournals.org/cgi/content/abstract/95/6/503

Reliability of genotyping is an issue for studies using non-invasive sources of DNA. We emphasize the importance of refining DNA extraction methods to maximize reliability and efficiency of genotyping for such DNA sources. We present a simple and general method to quantitatively compare genotyping reliability of various DNA extraction techniques and sample materials used. For bighorn sheep (Ovis canadensis) fecal samples we compare different fecal pellet materials, different amounts of fecal pellet material, and the effects of eliminating two DNA extraction steps for four microsatellite loci and four samples heterozygous at each locus. We evaluated 192 PCR outcomes for each treatment using indices of PCR success and peak height (signal strength) developed from analysis output of sequencer chromatograms. Outermost pellet material produced PCR results almost equivalent to DNA extracted from blood. Where any inner pellet material was used for DNA extraction, PCR results were poorer and inconsistent among samples. PCR success was not sensitive to amount of pellet material used until it was decreased to 15 mg from 60 mg. Our PCR index provides considerably more information relative to potential genotyping errors than simply comparing genotypes derived from paired fecal and blood or tissue samples. Our DNA extraction method probably has wide applicability to herbivores that produce pelleted feces where samples dry rapidly after deposition.


http://jhered.oupjournals.org/cgi/content/abstract/93/1/58

Black walnut (Juglans nigra L) is a large tree, native to the eastern United States, that is prized for its high-quality timber and edible nut. Thirty (GA/CT)n nuclear microsatellite markers were
identified from black walnut for use in population genetic studies, genome mapping, DNA
genotyping of important clones, studies of gene flow, and tree breeding. The markers were
polymorphic based on a diversity panel of 10 black walnut individuals from eight Midwestern U.S.
states.

Wolf, P. G., B. Doche, et al. (2004). "Genetic Structure of Rhododendron ferrugineum at a Wide Range of
Spatial Scales." J. Hered. 95(4): 301-308.
http://jhered.oupjournals.org/cgi/content/abstract/95/4/301

Rhododendron ferrugineum L. (Ericaceae) is a subalpine shrub found throughout the Pyrenees
and Alps at elevations of 1600-2200 m. We examined relationships between genetic and
geographic distance, using 115 dominant amplified fragment length polymorphism (AFLP)
markers to assess genetic structure over a wide range of spatial scales. We sampled 17 sites
with distances of 4 km to more than 1000 km between them. At these scales we detected no
association between geographic distance and genetic distance between populations. This
suggests that genetic drift and gene flow are not in equilibrium for these populations. This pattern
could have resulted from recent and rapid postglacial colonization, from more recent human
disturbance, or as a function of frequent and random "natural" long-distance colonization. At two
of our sites we used transects (two horizontal and two vertical with respect to slope at each site)
to sample at distances ranging from 10 m to more than 5000 m. At this scale we observed a
positive relationship between genetic and spatial distance along two vertical transects, one at
each site. We hypothesize that isolation-by-distance at this smaller scale is a function of restricted
gene flow via seed dispersal.

Common Morning Glory (Ipomoea purpurea)." J. Hered. 94(6): 442-448.
http://jhered.oupjournals.org/cgi/content/abstract/94/6/442

The common morning glory (Ipomoea purpurea) is highly polymorphic for flower color. Part of this
phenotypic variation is due to allelic variation at the P locus. This locus determines whether
flowers will be purple or pink, where purple is dominant to pink. We have determined that the
anthocyanin biosynthetic gene flavonoid 3'-hydroxylase (f3'h) corresponds to the P locus. In the
pink allele of f3'h there is a large insertion in the third exon, which results in the production of a
truncated transcript. This shortened transcript produces a nonfunctional F3'H enzyme, resulting in
the production of pink flowers rather than purple. In addition, we describe a polymerase chain
reaction (PCR)-based assay that can be used to determine the genotype of a plant at this locus.

J. Histochem. Cytochem. (11)

Multiple Functions for this ABC Transporter." J. Histochem. Cytochem. 51(7): 887-902.
http://www.jhc.org/cgi/content/abstract/51/7/887
We have studied the tissue distribution of Abcc6, a member of the ABC transmembrane transporter subfamily C, in normal C57BL/6 mice. RNase protection assays revealed that although almost all tissues studied contained detectable levels of the mRNA encoding Abcc6, the highest levels of Abcc6 mRNA were found in the liver. In situ hybridization (ISH) demonstrated abundant Abcc6 mRNA in epithelial cells from a variety of tissues, including hepatic parenchymal cells, bile duct epithelia, kidney proximal tubules, mucosa and gland cells of the stomach, intestine, and colon, squamous epithelium of the tongue, corneal epithelium of the eye, keratinocytes of the skin, and tracheal and bronchial epithelium. Furthermore, we detected Abcc6 mRNA in arterial endothelial cells, smooth muscle cells of the aorta and myocardium, in circulating leukocytes, lymphocytes in the thymus and lymph nodes, and in neurons of the brain, spinal cord, and the specialized neurons of the retina. Immunohistochemical analysis using a polyclonal Abcc6 rabbit antibody confirmed the tissue distribution of Abcc6 suggested by our ISH studies and revealed the cellular localization of Abcc6 in the basolateral plasma membrane in the epithelial cells of proximal convoluted tubules in the kidney. Although the function of Abcc6 is unknown, mutations in the human ABCC6 gene result in a heritable disorder of connective tissue called pseudoxanthoma elasticum (PXE). Our results demonstrating the presence of Abcc6 in epithelial and endothelial cells in a variety of tissues, including those tissues affected in PXE patients, suggest a possible role for Abcc6 in the normal assembly of extracellular matrix components. However, the presence of Abcc6 in neurons and leukocytes, two cell populations not associated with connective tissue, also suggests a more complex multifunctional role for Abcc6. (J Histochem Cytochem 51:887-902, 2003)


http://www.jhc.org/cgi/content/abstract/53/3/301
We assessed the feasibility of fetal RHD and RHCE genotyping by analysis of DNA extracted from plasma samples of RhD-negative pregnant women using real-time PCR and primers and probes targeted toward RHD and RHCE genes. We analyzed 45 pregnant women in the 11th to 40th weeks of pregnancy and correlated the results with serological analysis of cord blood after delivery. Non-invasive prenatal fetal RHD exon 7, RHD exon 10, RHCE exon 2 (C allele), and RHCE exon 5 (E allele) genotyping analysis of maternal plasma samples was correctly performed in 45 out of 45 RhD-negative pregnant women delivering 24 RhD-, 17 RhC-, and 7 RhE-positive newborns. Detection of fetal RHD and the C and E alleles of RHCE gene from maternal plasma is highly accurate and enables implementation into clinical routine. We recommend performing fetal RHD and RHCE genotyping together with fetal sex determination in alloimmunized D-negative pregnancies at risk of hemolytic disease of the newborn. In case of D-negative fetus, amplification of another paternally inherited allele (SRY and/or RhC and/or RhE positivity) proves the presence of fetal DNA in maternal circulation. (J Histochem Cytochem 53:301-305, 2005)


http://www.jhc.org/cgi/content/abstract/52/8/1011

Nucleoside reverse transcriptase inhibitors (NRTIs) have been a mainstay in the treatment of human immunodeficiency virus since the introduction of azidothymidine (AZT) in 1987. However, none of the current therapies can completely eradicate the virus, necessitating long-term use of anti-retroviral drugs to prevent viral re-growth. One of the side effects associated with long-term use of NRTIs is mitochondrial toxicity stemming from inhibition of the mitochondrial DNA (mtDNA) polymerase (gamma), which leads to mtDNA depletion and consequently to mitochondrial dysfunction. Here we report the use of fluorescence in situ hybridization (FISH) and immunocytochemistry (ICC) to monitor mtDNA depletion in cultured fibroblasts treated with the NRTI 2',3'-dideoxycytidine (ddC). These techniques are amenable to both microscopy and flow cytometry, allowing analysis of populations of cells on a single-cell basis. We show that, as mtDNA depletion progresses, a mosaic population develops, with some cells being depleted of and others retaining mtDNA. These techniques could be useful as potential therapeutic monitors to indicate when NRTI therapy should be interrupted to prevent mitochondrial toxicity and could aid in the development of less toxic NRTIs by providing an assay suitable for pharmacodynamic evaluation of candidate molecules. (J Histochem Cytochem 52:1011-1018, 2004)


http://www.jhc.org/cgi/content/abstract/53/2/155

Endothelial nitric oxide synthase (eNOS), the major nitric oxide (NO)-generating enzyme of the vasculature, is regulated through multiple interactions with proteins, including caveolin-1, Hsp90, Ca2+-calmodulin, and the recently discovered eNOS-interacting protein, NOSIP. Previous studies indicate that NOSIP may contribute to the intricate regulation of eNOS activity and availability. Because eNOS has been shown to be abundantly expressed in the airways, we determined the expression and cellular localization of NOSIP in rat trachea and lung by RT-PCR and immunohistochemistry and examined the interaction of NOSIP with eNOS in lung by coimmunoprecipitation. In tracheal epithelium and lung, NOSIP mRNA expression was prevalent, as shown by RT-PCR, and the corresponding protein interacted with eNOS, as demonstrated by coimmunoprecipitation. Using immunohistochemistry, we found both NOSIP and eNOS immunoreactivity in ciliated epithelial cells of trachea and bronchi, while Clara cells showed
immunoreactivity for NOSIP only. NOSIP and eNOS were present in vascular and bronchial smooth muscle cells of large arteries and airways, whereas endothelial cells, as well as bronchiolar and arteriolar smooth muscle cells, exclusively stained for NOSIP. Our results point to functional role(s) of NOSIP in the control of airway and vascular diameter, mucosal secretion, NO synthesis in ciliated epithelium, and, therefore, of mucociliary and bronchial function. (J Histochem Cytochem 53:155-164, 2005)


http://www.jhc.org/cgi/content/abstract/51/12/1645

The arterial vascular wall contains a non-neuronal intrinsic cholinergic system. The rate-limiting step in acetylcholine (ACh) synthesis is choline uptake. A high-affinity choline transporter, CHT1, has recently been cloned from neural tissue and has been identified in epithelial cholinergic cells. Here we investigated its presence in rat and human arteries and in primary cell cultures of rat vascular cells (endothelial cells, smooth muscle cells, fibroblasts). CHT1-mRNA was detected in the arterial wall and in all isolated cell types by RT-PCR using five different CHT1-specific primer pairs. Antisera raised against amino acids 29-40 of the rat sequence labeled a single band (50 kD) in Western blots of rat aorta, and an additional higher molecular weight band appeared in the hippocampus. Immunohistochemistry demonstrated CHT1 immunoreactivity in endothelial and smooth muscle cells in situ and in all cultured cell types. A high-affinity [3H]-choline uptake mechanism sharing characteristics with neuronal high-affinity choline uptake, i.e., sensitivity to hemicholinium-3 and dependence on sodium, was demonstrated in rat thoracic aortic segments by microimager autoradiography. Expression of the high-affinity choline transporter CHT1 is a novel component of the intrinsic non-neuronal cholinergic system of the arterial vascular wall, predominantly in the intimal and medial layers. (J Histochem Cytochem 51:1645-1654, 2003)


http://www.jhc.org/cgi/content/abstract/52/11/1483

We fractionated leukocytes from three donors into >90% pure samples of granulocytes, lymphocytes, and monocytes and tested them for transcriptional and translational expression of three physiologically-proven lactate transporters, monocarboxylate transporter 1 (MCT1), MCT2, and MCT4, using RT-PCR and affinity-purified rabbit antibody (Ab) to the C-terminal segment of each human MCT. Transcripts of all three MCTs were identified in each leukocyte fraction by RT-PCR and proven by sequencing of fragments extracted after isolation on agarose gels. Transporter protein of the appropriate size was demonstrated for each of the monocarboxylate transporters MCTs in lymphocytes and monocytes by Western blot, while lower-molecular-weight bands were found in granulocytes and are presumed to be degraded forms, because they were blocked by antibody-antigen (Ab-Ag) preincubation. IHC demonstrated all three MCTs in methanol-fixed droplets of all three leukocyte fractions; stain was abolished on omission of the primary Ab. Plasmalemmal staining occurred with all MCTs in all leukocyte fractions. Because the Km for lactate increases approximately fivefold at each step, with MCT2<1<4, leukocytes must use the full range of lactate binding to survive in acidic and hypoxic environments. Except for MCT4 in lymphocytes, all the MCTs also stained leukocyte cytoplasm, often with distinct granularity. Nuclear membrane staining was also seen with MCT1 and MCT2, while platelet plasmalemma stained only with MCT2. (J Histochem Cytochem 52:1483-1493, 2004)
Bilateral neurectomy of the pelvic nerve (BLPN) that carries uterine cervix-related sensory nerves induces dystocia, and administration of its vasoactive neuropeptides induces changes in the cervical microvasculature, resembling those that occur in the ripening cervix. This study was designed to test the hypothesis that (a) the cervix of pregnant rats expresses vascular endothelial growth factor (VEGF) and components of the angiogenic signaling pathway [VEGF receptors (Flt-1, KDR), activity of protein kinase B, Akt (phosphorylated Akt), and endothelial nitric oxide synthase (eNOS)] and von Willebrand Factor (vWF) and that these molecules undergo changes with pregnancy, and (b) bilateral pelvic neurectomy (BLPN) alters levels of VEGF concentration in the cervix. Using RT-PCR and sequencing, two VEGF isoforms, 120 and 164, were identified in the rat cervix. VEGF, VEGF receptor-1 (Flt-1), eNOS, and vWF immunoreactivities (ir) were localized in the microvasculature of cervical stroma. Their protein levels increased during pregnancy but decreased to control levels by 2 days postpartum. VEGF receptor-2 (KDR)-ir was confined to the epithelium of the endocervix. BLPN downregulated levels of VEGF by a third. Therefore, the components of the angiogenic signaling pathway are expressed in the cervix and change over pregnancy. Furthermore, angiogenic and sensory neuronal factors may be important in regulating the dynamic microvasculature in the ripening cervix and may subsequently play a role in cervical ripening and the birth process. (J Histochem Cytochem 52:1665-1674, 2004)

We have previously demonstrated by immunohistochemistry the presence of secreted carbonic anhydrase (CA VI) in the acinar cells of the rat lacrimal glands. In this study we purified the sheep lacrimal gland CA VI to homogeneity and demonstrated by Western analysis that it has the same apparent subunit molecular weight (45 kD) as the enzyme isolated from saliva. RT-PCR analysis showed that CA VI mRNA from the lacrimal gland was identical to that of the parotid gland CA VI mRNA. An RIA specific for sheep CA VI showed the lacrimal gland tissue concentration of the enzyme to be 4.20 {+/-} 2.60 ng/mg protein, or about 1/7000 of the level found in the parotid gland. Immunohistochemistry (IHC) and in situ hybridization (ISH) showed that lacrimal acinar cells expressed both immunoreactivity and mRNA for CA VI. Moreover, CA VI immunoreactivity was occasionally observed in the lumen of the ducts. Unlike the parotid gland, in which all acinar cells expressed CA VI immunoreactivity and mRNA, only some of the acinar cells of the lacrimal gland showed expression. These results indicate that the lacrimal gland synthesizes and secretes a very small amount of salivary CA VI. In tear fluid, CA VI is presumed to have a role in the maintenance of acid/base balance on the surface of the eye, akin to its role in the oral cavity. (J Histochem Cytochem 50:821-827, 2002)
To elucidate the behavior of autologously transplanted mesenchymal cells in osteochondral defects, we followed transplanted cells using green fluorescent protein (GFP) transgenic rats, in which all cells express GFP signals in their cytoplasm and nuclei as transplantation donors. Bone marrow-derived mesenchymal cells, which contain mesenchymal stem cells (MSCs), were obtained from transgenic rats. Then, dense mesenchymal cell masses created by hanging-drop culture were transplanted and fixed with fibrin glue into osteochondral defects of wild-type rats. At 24 weeks after surgery, the defects were repaired with hyaline-like cartilage and subchondral bone. GFP positive cells, indicating transplanted mesenchymal-derived cells, were observed in the regenerated tissues for 24 weeks although GFP positive cells decreased in number with time. Because GFP causes no immunological rejection and requires no chemicals for visualization, transplantation between transgenic and wild-type rats can be regarded as a simulation of autologous transplantation, and the survivability of transplanted cells are able to be followed easily and reliably. Thus, the behavior of transplanted mesenchymal cells was able to be elucidated in vivo by this strategy, and the results could be essential in future tissue engineering for the regeneration of osteochondral defects with original hyaline cartilage and subchondral bone. (J Histochem Cytochem 53:207-216, 2005)


During the course of diagnostic surgical pathology, pathologists have established a large collection of formalin-fixed, paraffin-embedded tissues that form invaluable resources for translational studies of cancer and a variety of other diseases. Accessibility of macromolecules in the fixed tissue specimens is a critical issue as exemplified by heat-induced antigen retrieval (AR) immunohistochemical (IHC) staining. On the basis of observations that heating may also enhance in situ hybridization (ISH) and the similarity of formalin-induced chemical modifications that occur in protein and in DNA, we designed a study to examine the efficiency of DNA extraction from archival formalin-fixed, paraffin-embedded tissues using an adaptation of the basic principles of the AR technique, i.e., heating the tissue under the influence of different pH values. Archival paraffin blocks of lymph nodes, tonsil, and colon were randomly selected. Each paraffin block was prepared in 34 microtubes. For each paraffin block, one tube was used as a control sample, using a non-heating DNA extraction protocol. The other 33 tubes were tested using a heating protocol under 11 variable pH values (pH 2 to 12) under three different heating conditions (80, 100, and 120C). Evaluation of the results of DNA extraction was carried out by measuring yields by photometry and PCR amplification, as well as kinetic thermocycling (KTC)-PCR methods. In general, lower pH (acid) solutions gave inferior results to solutions at higher pH (alkaline). Heating tissues at a higher temperature and at pH 6-9 gave higher yields of DNA. There appeared to be a peak in terms of highest efficiency of extracted DNA at around pH 9. The average ratios 260:280 of extracted DNA also showed better values for samples heated at 120C. PCR products of three primers showed satisfactory results for DNA extracted from archival paraffin-embedded tissues by heating protocols at pH 6-12, with results that were comparable to the control sample subjected to the standard non-heating, enzymatic DNA extraction method. This study is the first to document the use of heating at an alkaline pH for DNA extraction from archival formalin-fixed, paraffin-embedded tissues, a recommendation based on the principles of AR for protein IHC. These findings may lead to a more effective protocol for DNA extraction from archival paraffin-embedded tissues and may also provide enhanced understanding of changes that occur during formalin-induced modification of nucleic acids. (J Histochem Cytochem 50:1005-1011, 2002)
Eicosanoids generated via cyclooxygenase-2 (COX-2) and nitric oxide produced from inducible nitric oxide synthase (NOSII) have been implicated in endotoxin-induced tissue injury. In the present studies, we characterized COX-2 and NOSII activity in rat hepatic macrophages and their interaction during acute endotoxemia. Kupffer cells from control animals were found to constitutively express COX-2 and NOSII mRNA and protein. Whereas treatment of the cells with lipopolysaccharide (LPS) and/or interferon-γ (IFN-γ) had no major effect on COX-2, NOSII expression increased. Induction of acute endotoxemia resulted in a rapid and transient increase in constitutive COX-2 expression and prostaglandin E2 (PGE2) production by liver macrophages as well as NOSII expression and nitric oxide release. Cells from endotoxin-treated rats were also sensitized to generate more nitric oxide and express increased NOSII in response to LPS and IFN-γ. Inhibition of NOSII with aminoguanidine reduced COX-2 mRNA and protein expression as well as PGE2 production by activated macrophages from endotoxemic, but not control animals. In contrast, SC236, a specific COX-2 inhibitor, had no effect on NOSII mRNA or protein levels or on nitric oxide production by hepatic macrophages, even after endotoxin administration. These data suggest that activation of COX-2 may be important in the pathophysiological response of hepatic macrophages to endotoxin. Moreover, nitric oxide is involved in regulating COX-2 in activated liver macrophages during acute endotoxemia.

Regulation of humoral responses involves multiple cell types including the requirements for cognate interactions between T and B cells to drive CD40-dependent responses to T-dependent antigens. A third cell type has also been shown to play an essential role, the dendritic cell (DC). We demonstrate that bovine peripheral blood-derived (PB)-DC are similar in function to features described for human interstitial DC including the production of signature type 2 cytokines [interleukin (IL)-13, IL-10]. PB-DC express moderate-to-high costimulatory molecule expression, and major histocompatibility complex class II is negative for CD14 expression and has low or no expression of CD11c. Consistent with the interstitial phenotype is the ability of PB-DC to influence B cell activation and differentiation via direct expression of CD40L and type 2 cytokines. Collectively, these results suggest that direct B cell-DC interactions may promote an immunoglobulin-isotype expression pattern consistent with type 2 responses, independent of direct T cell involvement.

Malignant pleural mesothelioma is a highly aggressive tumor arising from the mesothelial cells that line the pleural cavities. This tumor is resistant to most conventional anticancer treatments and appears to be very sensitive to growth-promoting influences of cytokines and growth factors. Identification of natural inhibitory pathways that control growth should aid discovery of novel therapeutic approaches. We hypothesized that (alpha)-melanocyte-stimulating hormone ((alpha)-MSH), which is produced by many cell types and antagonizes cytokines and growth factors, could be an endogenous inhibitory molecule in mesothelioma. Twelve mesothelioma cell lines were established from pleural effusions of patients with malignant mesothelioma. Mesothelioma cells were found to express mRNA for proopiomelanocortin and its processing enzymes; release (alpha)-MSH peptide into supernatants; and express melanocortin 1 receptor (MC1R), the high-affinity receptor for (alpha)-MSH. Immunoneutralization of MC1R in the cell lines enhanced expression of interleukin-8 (IL-8), IL-6, and transforming growth factor-{beta}. These molecules promote mesothelioma proliferation and are considered therapeutic targets in this tumor. Coincubation of mesothelioma cells with synthetic (alpha)-MSH significantly reduced cell proliferation. The present research shows an autocrine-inhibitory circuit based on (alpha)-MSH and its receptor MC1R. Activation of MC1R by selective peptides or peptidomimetics might provide a novel strategy to reduce mesothelioma cell proliferation by taking advantage of this endogenous inhibitory circuit.


We report that mitochondrial DNA (mtDNA) is inflammatogenic in vitro and in vivo as a result of the presence of unmethylated CpG sequences and its oxidative status. Purified human and murine mtDNAs induced arthritis when injected intra-articularly (i.a.) in mice. Importantly, oligodeoxynucleotide that contained a single oxidatively damaged base also induced arthritis when injected i.a. in mice. In contrast, neither human nor murine nuclear DNA induced inflammation. mtDNA-induced arthritis was neither B cell-nor T cell-dependent but was mediated by monocytes/macrophages. mtDNA-induced nuclear factor-{kappa}B stimulation resulted in the production of tumor necrosis factor (alpha), a potent, arthritogenic factor. Finally, extracellular mtDNA was detected in the synovial fluids of rheumatoid arthritis patients but not of control subjects. We conclude that endogenous mtDNA displays inflammatogenic properties as a result of its content of unmethylated CpG motifs and oxidatively damaged adducts.


Copines are a recently identified group of proteins characterized by two Ca2+-binding C2-domains at the N terminus and an A-domain at the C terminus. Although pEST sequences indicate the existence of at least seven copines in man, only copines I, III, and VI have been identified at protein level. Here, we describe the isolation of copines I and III in the cytosol of human neutrophils by use of Ca2+-induced hydrophobic chromatography. This is the first demonstration that copines are coexpressed in the same cell. We found that copine III exists in the cytosol of human neutrophils as a monomer with a blocked N terminus. Copines I and III undergo conformational changes upon Ca2+ binding that lead to exposure of hydrophobic
patches. Examination of RNA from 68 human tissues demonstrated that copines I-III are ubiquitously expressed whereas copines IV-VII each has a more restricted and individual expression profile. Expression of copines I-III was also demonstrated in neutrophil precursors from bone marrow. Copine I was uniformly expressed at all stages of neutrophil differentiation, whereas copine II and even more so, copine III were expressed in the more immature neutrophil precursors, which indicates an individual function of these copines.


http://www.jleukbio.org/cgi/content/abstract/75/5/836

In the present study, we investigated the molecular mechanisms of spontaneous and tumor necrosis factor \(\text{(alpha)}\) (TNF-\(\text{(alpha)}\))-mediated apoptosis of human polymorphonuclear neutrophils (PMN). Whereas TNF-\(\text{(alpha)}\)-mediated apoptosis was almost absent in the presence of the caspase-8 inhibitor Z-Ac-Ala-Glu-Val-Asp-7-fluoromethyl ketone (Z-DEVD-FMK), the inhibitor had no effect on spontaneous apoptosis, suggesting that spontaneous apoptosis was independent of caspase-8. Subsequently, we identified different isoforms of caspase-10 in human PMN and found high expression of caspase-10/b and/or -10/d and low expression of caspase-10/a and -10/c at the mRNA level. At the protein level, freshly isolated PMN showed high expression of caspase-10/b and -10/d as well as moderate expression of caspase-10/a and -10/c. Upon spontaneous apoptosis, caspase-10/b was down-regulated, which was accompanied by the appearance of a specific 47-kDa caspase-10/b cleavage product and an increased caspase-10 activity. In contrast, no down-regulation of caspase-10/a, -10/c, or -10/d was observed, suggesting that spontaneous apoptosis was associated with a differential activation of caspase-10/b. This was confirmed by the finding that spontaneous apoptosis was inhibited in the presence of Z-Ile-Glu-Thr-Asp (Z-IETD)-FMK, which blocks caspase-10. However, no down-regulation of caspase-10 isoforms was observed in the presence of TNF-\(\text{(alpha)}\), suggesting that caspase-10 was not involved in TNF-\(\text{(alpha)}\)-induced apoptosis. Taken together, our study demonstrates that spontaneous and TNF-\(\text{(alpha)}\)-mediated apoptosis of PMN have different molecular requirements. Whereas TNF-\(\text{(alpha)}\)-mediated apoptosis depends on the activation of caspase-8, spontaneous apoptosis requires the activation of caspase-10/b. This finding may reveal that PMN apoptosis in different (patho-) physiological settings results from distinct molecular mechanisms.

Iwamoto, S., M. Ishida, et al. (2005). "Lipopolysaccharide stimulation converts vigorously washed dendritic cells (DCs) to nonexhausted DCs expressing CD70 and evoking long-lasting type 1 T cell responses." J. Leukoc. Biol.: jlb.1104654.

http://www.jleukbio.org/cgi/content/abstract/jlb.1104654v1

A great variety of in vitro culture protocols for human monocyte-derived dendritic cells (mo-DCs) has been used to generate DCs suitable for use in immunotherapy. It is thought that activated DCs undergo one-way differentiation into "exhausted" DCs. In the present study, we contrived an in vitro method for facilitating expression of CD70 by mature DCs. This was achieved by vigorous washing of mo-DCs before exposure to lipopolysaccharide (LPS). Unexpectedly, these mature DCs retain expression of some interleukin (IL)-12 family members after extended periods and maintain their ability to stimulate type 1 T cell responses. In contrast, DCs exposed to IL-4 before LPS stimulation or LPS-stimulated DCs not exposed to washing stress before activation failed to express CD70 and did differentiate into exhausted DCs. It is interesting that DCs expressing CD70 (CD70+ DCs) induced interferon-\(\text{\{gamma\}}\) production from purified, allogeneic CD8+ T cells through a direct CD27-CD70 interaction. This is evidence for a pathway resulting in generation of CD8 T effectors by B7-independent mechanisms. These data suggest that
exposure of immature DCs to LPS stimulation contributes to their terminal differentiation into CD70+ DCs, which have potent ability to prolong type 1 T cell responses through alternative pathways.


http://www.jleukbio.org/cgi/content/abstract/76/5/1057

The calcineurin-dependent, cyclosporin A (CsA)-sensitive transcription factor nuclear factor of activated T cells (NFAT) represents a group of proteins, which is well-characterized as a central regulatory element of cytokine expression in activated T cells. In contrast, little is known about the expression or function of NFAT family members in myeloid cells; moreover, it is unclear whether they are expressed by hematopoietic stem/progenitor cells. Here, we show that NFATc2 (NFAT1) is expressed at high levels in CD34+ cells and megakaryocytes but not in cells committed to the neutrophilic, monocyctic, or erythroid lineages. Cytokine-induced in vitro differentiation of CD34+ cells into neutrophil granulocytes results in the rapid suppression of NFATc2 RNA and protein. NFATc2 dephosphorylation/rephosphorylation as well as nuclear/cytoplasmic translocation in CD34+ cells follow the same calcineurin-dependent pattern as in T lymphocytes, suggesting that NFATc2 activation in these cells is equally sensitive to inhibition with CsA. Finally, in vitro proliferation, but not differentiation, of CD34+ cells cultured in the presence of fms-like tyrosine kinase 3 ligand (FLT3L), stem cell factor, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-3, and G-CSF is profoundly inhibited by treatment with CsA in a dose-dependent manner. These results suggest a novel and unexpected role for members of the NFAT transcription factor family in the hematopoietic system.


http://www.jleukbio.org/cgi/content/abstract/73/3/369

To explore whether the proinflammatory products of the 5-lipoxygenase (5-LOX) pathway are involved in microglia-mediated toxicity toward neuronal cells, we evaluated the effects of 5-LOX inhibitors using an in vitro assay system where human neuronal SH-SY5Y cells are exposed to toxic secretions from THP-1 monocytic cells or human microglia. The specific 5-LOX inhibitors, REV 5901, zileuton, and 5-hydroxyeicosatetraenoic acid lactone; the nonselective LOX inhibitors, phenidone and dapsone; the dual 5-LOX/cyclooxygenase inhibitor, tepoxalin; and the selective inhibitor of the 5-LOX-activating protein (FLAP), MK-886, inhibited such toxicity. The toxicity was enhanced by the 5-LOX product leukotriene (LT)D4 and reduced by the selective cysteiny1 LT receptor (CysLT1) antagonist MK-571. The mRNAs for 5-LOX and FLAP were detected in THP-1 cells and human microglia but not in SH-SY5Y cells. The data suggest that inhibition of proinflammatory LT production by 5-LOX inhibition could selectively reduce toxicity of microglial cells and thus be beneficial in neuroinflammatory diseases.

Monocytes/macrophages in ischemic tissues are involved in inflammation and suppression of adaptive immunity via secretion of proinflammatory cytokines and reduced ability to trigger T cells, respectively. We subjected human mononuclear cells and mouse macrophages to hypoxia and reoxygenation, the main constituents of ischemia and reperfusion, and added lipopolysaccharide (LPS) to simulate bacterial translocation, which frequently accompanies ischemia. We monitored the secretion of tumor necrosis factor (alpha) (TNF-(alpha)) and the surface expression of human leukocyte antigen-DR and the costimulatory molecules CD80 and CD86 on monocytes/macrophages. Hypoxia selectively reduced the surface expression of CD80 (P<0.01), and synergistically with LPS, it enhanced TNF-(alpha) secretion (P<0.003).

Reoxygenation reversed both phenomena. In the mouse macrophage cell line RAW 264.7, hypoxia reduced the surface expression of CD80 and increased its concentrations in the supernatants (P<0.01). Down-regulation of the mRNA coding for the membrane-anchored CD80 was observed, suggesting that hypoxia triggers alternative splicing to generate soluble CD80.

Cumulatively, these results suggest that hypoxia simultaneously affects monocytes/macrophages to enhance inflammation and reduce their ability to initiate adaptive-immunity responses associated with ischemic injury.


Several lines of evidence have suggested that a CXC chemokine receptor 4 (CXCR4)/stromal cell-derived factor-1 (SDF-1; CXC chemokin ligand 12 (CXCL12)) pair is involved in baseline trafficking of leukocytes into extravascular tissues and that modulation of surface CXCR4 expression may represent an alternative mechanism for control of cell-specific biological responses to SDF-1/CXCL12. We explored the regulation of CXCR4 expression by cytokines in polymorphonuclear neutrophils (PMNs). No significant surface expression of CXCR4 in freshly isolated PMNs was detected, but expression became apparent gradually during incubation. SDF-1(α)/CXCL12 initiated Ca2+ mobilization and migratory responses in 20 h cultured PMNs. The surface CXCR4 expression was suppressed most potently by interferon-(gamma) (IFN-(gamma)). IFN-(alpha), granulocyte-macrophage colony-stimulating factor (GM-CSF), and G-CSF also inhibited spontaneous CXCR4 expression. Real-time, quantitative PCR experiments revealed that a spontaneous increase and an IFN-(gamma)-mediated decrease in surface CXCR4 paralleled changes in the CXCR4 mRNA level. These results on PMNs support the argument that the SDF-1 (CXCL12)/CXCR4 system is regulated by cell type-specific mechanisms.


The replacement of bone marrow (BM) as a conventional source of stem cell (SC) by umbilical cord blood (UCB) and granulocyte-colony stimulating factor-mobilized peripheral blood SC (PBSC) has brought about clinical advantages. However, several studies have demonstrated that UCB CD34+ cells and PBSC significantly differ from BM CD34+ cells qualitatively and quantitatively. Here, we quantified the number of SC in purified BM, UCB CD34+ cells, and
CD34+ PBSC using in vitro and in vivo assays for human hematopoietic SC (HSC) activity. A cobblestone area-forming cell (CAFC) assay showed that UCB CD34+ cells contained the highest frequency of CAFCw6 (3.6- to tenfold higher than BM CD34+ cells and PBSC, respectively), and the engraftment capacity in vivo by nonobese diabetic/severe combined immunodeficiency repopulation assay was also significantly greater than BM CD34+, with a higher proportion of CD45+ cells detected in the recipients at a lower cell dose. To understand the molecular characteristics underlying these functional differences, we performed several DNA microarray experiments using Affymetrix gene chips, containing 12,600 genes. Comparative analysis of gene-expression profiles showed differential expression of 51 genes between BM and UCB CD34+ SC and 64 genes between BM CD34+ cells and PBSC. These genes are involved in proliferation, differentiation, apoptosis, and engraftment capacity of SC. Thus, the molecular expression profiles reported here confirmed functional differences observed among the SC sources. Moreover, this report provides new insights to describe the molecular phenotype of CD34+ HSC and leads to a better understanding of the discrepancy among the SC sources.


In our study of the modulation of the expression of inflammation-related genes in neutrophils, we have found a gene called CLECSF6 (C-type lectin superfamily 6). CLECSF6 expresses two mRNA species at low levels in resting neutrophils. Here, we describe for the first time the sequence of the short mRNA version. It lacks amino acids that are likely to affect the functionality of its protein product. GM-CSF, IL-3, IL-4, and IL-13 caused an accumulation of the short CLECSF6 mRNA in neutrophils. The surface expression of the CLECSF6 protein was reduced by TNF-\(\alpha\), IL-1\(\alpha\), LPS, and Matrigel(R). CLECSF6 bears the immunoreceptor tyrosine-based inhibition motif (ITIM) involved in signal transduction resulting in the inhibition of leukocyte activation. We propose that some neutrophil activators modulate the expression of CLECSF6 at the mRNA (GM-CSF, IL-3, IL-4, and IL-13) or protein (TNF-\(\alpha\), IL-1\(\alpha\), LPS, and Matrigel(R)) levels in ways that block ITIM-based transduction of anti-inflammatory signals and therefore promote inflammation.


Complex syndromes such as atherosclerosis and type 2 diabetes are disorders that are associated with inflammatory processes involving innate and adaptive immunity. Emerging knowledge about the pathological consequences of immune imbalances in a wide range of disease settings is expected to help to identify novel therapeutic targets. However, current test systems for immunomodulatory drugs tend to be too simplistic, as they rely only on cells of the innate- or the adaptive-immune system, or they are complex, in vivo models, which are not suitable for screening purposes. Using a modified mixed lymphocyte culture (MMLC) assay for combined analysis of innate and adaptive immunity, we show that this assay is very sensitive for the presence of low concentrations of immunomodulatory agents. Low-dose lipopolysaccharide stimulation of cells from two unrelated donors yields a strong cytokine response including interleukin (IL)-12 and IL-18, which induce interferon-\(\gamma\) as a potential analysis parameter. As the MMLC assay is based on the mutual interaction of cells of the innate and adaptive immunity, it enables the monitoring of cytokine release under almost physiological conditions and might be of interest for the characterization of known and novel drugs concerning their
Human plasmacytoid or CD4+CD11c- type 2 dendritic cell precursors (PDC) were identified as natural type I interferon (IFN)-producing cells in response to viral and bacterial infection. They represent effector cells of innate immunity and link it to the distinct adaptive immunity by differentiating into mature DC. It has been reported that oligodeoxyribonucleotides containing unmethylated CpG motifs (CpG DNA) stimulate PDC to produce IFN-α, but the molecular mechanisms involved remain unknown. We found that CpG-DNA-induced IFN-α production in PDC was completely impaired by the inhibitor of the p38 mitogen-activated protein kinase (MAPK) pathway. Expression of IFN regulatory factor (IRF)-7 was enhanced by CpG-DNA treatment, which was preceded by the phosphorylation of signal transducer and activator of transcription (STAT)1 on Tyr-701, as well as its enhanced phosphorylation on Ser-727. All of these events were also suppressed by the p38 MAPK inhibitor. STAT1, STAT2, and IRF-9, components of IFN-stimulated gene factor 3 (ISGF3), were recognized in the nuclear fraction of CpG-DNA-treated cells. Neither anti-IFN-α/β antibodies (Ab) nor anti-IFNAR Ab suppressed STAT1 phosphorylation, enhancement of IRF-7 expression, or IFN-α production in the early phase of the culture. These results suggest that CpG DNA induces p38 MAPK-dependent phosphorylation of STAT1 in a manner independent of IFN-α/β, which may cause ISGF3 formation to increase the transcription of the IRF-7 gene, thereby leading to IFN-α production in human PDC.

Epithelia- and leukocyte-associated antimicrobial peptides provide immediate protection against microbial infections by rapidly inactivating potential pathogens. Bac5 is a member of the cathelicidin family of antimicrobial peptides and is stored in the cytoplasmic granules of bovine neutrophils. We investigated the expression of this gene in airway and intestine, and although the gene was not found to be locally expressed in these tissues, a strong Bac5 induction signal was detected by in situ hybridization in neutrophils infiltrating infected lung, consistent with expression of this gene in activated neutrophils. The Bac5 gene was also induced in bovine peripheral neutrophils stimulated with Escherichia coli or purified lipopolysaccharide (LPS) but not in other blood cells and in resting neutrophils. The levels of Bac5 mRNA increased at 12-24 h post-stimulation, and a dose-dependent increase in Bac5 expression was determined in the presence of increasing amounts of LPS. A metabolically labeled product with a molecular weight compatible with that of proBac5 was immunoprecipitated from cell-free media of stimulated neutrophils, suggesting that the newly synthesized polypeptide is released extracellularly. Collectively, these results provide the first evidence that fully differentiated neutrophils are capable of de novo synthesis and secretion of a granule-associated antimicrobial peptide.
Granulocyte-colony stimulating factor (G-CSF) induces proliferation of myeloid progenitor cells and controls their differentiation into mature neutrophils. Signal transducer and activator of transcription (STAT) proteins STAT3 and STAT5 are activated by G-CSF and play distinct roles in neutrophil development. Suppressor of cytokine signaling (SOCS) proteins are induced by STATs and inhibit signaling through various negative-feedback mechanisms. SOCS proteins can compete with docking of signaling substrates to receptors, interfere with Janus tyrosine kinase activity, and target proteins for proteasomal degradation. The latter process is mediated through the conserved C-terminal SOCS box. We determined the role of various SOCS proteins in controlling G-CSF responses and investigated the involvement of the SOCS box therein. We show that SOCS1 and SOCS3, but not CIS and SOCS2, inhibited G-CSF-induced STAT activation in human embryo kidney 293 cells. In myeloid 32D cells, SOCS1 and SOCS3 are induced by G-CSF. However, relative to interleukin-3-containing cultures, during G-CSF-induced neutrophilic differentiation, SOCS3 expression was further elevated, while SOCS1 levels remained constant. SOCS box deletion mutants of SOCS1 and SOCS3 were severely hampered in their abilities to inhibit STAT activation and to efficiently suppress colony formation by primary myeloid progenitors in response to G-CSF. These data demonstrate the importance of the SOCS box for the inhibitory effects of SOCS proteins on G-CSF signaling and show that among the different SOCS family members, SOCS3 is the major negative regulator of G-CSF responses during neutrophilic differentiation.


Wild-type and {micro}-opioid receptor knockout (MORKO) mice were used to investigate the role of corticosterone (CORT) and the {micro}-opioid receptor (MOR) in chronic morphine-mediated immunosuppression. We found that although plasma CORT concentrations in CORT infusion (10 mg/kg/day) and morphine-pellet implantation (75 mg) mice were similar (400-450 ng/ml), chronic morphine treatment resulted in a significantly higher (two- to threefold) inhibition of thymic, splenic, and lymph node cellularity; inhibition of thymic-lymphocyte proliferation; inhibition of IL-2 synthesis; and activation of macrophage nitric oxide (NO) production when compared with CORT infusion. In addition, results show that the inhibition of IFN-{{gamma}} synthesis and splenic- and lymph node-lymphocyte proliferation and activation of macrophage TNF-{{alpha}} and IL-1{{beta}} synthesis occurred only with chronic morphine treatment but not with CORT infusion. These morphine effects were abolished in MORKO mice. The role of the sympathetic nervous system on morphine-mediated effects was investigated by using the ganglionic blocker chlorisondamine. Our results show that chlorisondamine was able to only partially reverse morphine's inhibitory effects. The results clearly show that morphine-induced immunosuppression is mediated by the MOR and that although some functions are amplified in the presence of CORT or sympathetic activation, the inhibition of IFN-{{gamma}} synthesis and activation of macrophage-cytokine synthesis is CORT-independent and only partially dependent on sympathetic activation.

{gamma}{delta} T-lymphocytes are believed to play a role in maintaining the normal configuration of epithelial tissue. As little is known about the factors mediating this function, we addressed the question of whether {gamma}{delta} T-lymphocytes produce fibroblast growth factor (FGF)-9 as well as two other growth factors associated with epithelial tissue reconstitution. Blood {gamma}{delta} T cells isolated from healthy donors were grown in the presence of isopentenyl pyrophosphate (IPP) or transforming growth factor-{beta}1 (TGF-{beta}1)/interleukin-15 (IL-15) for 24 h and were assessed for the expression and synthesis of FGF-9, keratinocyte growth factor (KGF), and epidermal growth factor (EGF). Resting human {gamma}{delta} T cells constitutively expressed KGF and FGF-9 mRNA but no EGF mRNA. In the presence of IPP, FGF-9 mRNA expression significantly increased in a dose-dependent manner, expression of KGF remained unaltered, and EGF mRNA could not be detected. In contrast to IPP, stimulation of the cells with TGF-{beta}1/IL-15 did not alter FGF-9 expression. Moreover, stimulation with anti-CD3 does not induce FGF-9 expression but triggers a high signal of interferon-{gamma} mRNA. Western blot analysis of {gamma}{delta} T cell lysates, prepared 4 days following stimulation with IPP, showed an increase of FGF-9 protein as compared with control cells. In conclusion, the results demonstrate for the first time that human blood and bronchoalveolar lavage {gamma}{delta} T-lymphocytes are capable of expressing FGF-9. The data also provide novel evidence that immunoregulatory cells can synthesize FGF-9.


The MUC1 mucin (CD227) is a cell surface mucin originally thought to be restricted to epithelial tissues. We report that CD227 is expressed on human blood dendritic cells (DC) and monocyte-derived DC following in vitro activation. Freshly isolated murine splenic DC had very low levels of CD227; however, all DC expressed CD227 following in vitro culture. In the mouse spleen, CD227 was seen on clusters within the red pulp and surrounding the marginal zone in the white pulp. Additionally, we confirm CD227 expression by activated human T cells and show for the first time that the CD227 cytoplasmic domain is tyrosine-phosphorylated in activated T cells and DC and is associated with other phosphoproteins, indicating a role in signaling. The function of CD227 on DC and T cells requires further elucidation.

J. Lipid Res. 6


The aim of this study was first to examine the relationships between adiponectin gene (Apm1) polymorphisms and anthropometric indices as well as plasma adiponectin and lipoprotein/lipid levels, and then to investigate whether the presence of visceral obesity or insulin resistance may modulate the impact of these polymorphisms on metabolic risk variables. Molecular screening of
the Apm1 gene was achieved, and a sample of 270 unrelated men recruited from the greater Quebec City area and selected to cover a wide range of body fatness values was genotyped. Sequencing of the Apm1 gene revealed two previously reported polymorphisms (c.45T>G and c.276G>T) as well as two newly identified genetic variations (-13752delT and -13702G>C). Carriers of the c.276T allele had higher LDL-cholesterol and lower HDL-triglyceride concentrations than did 276G/G homozygotes (P = 0.02 and P = 0.01, respectively). Carriers of the c.45G allele exhibited higher plasma adiponectin concentrations than did 45T/T homozygotes (P = 0.04). After dividing each genotype group into subgroups for visceral AT, homozygotes for the normal allele at position -13752delT, carriers of the c.45G allele, and carriers of the c.276T allele had similar total apolipoprotein B (apoB) concentrations, whether they were viscerally obese or not. These results suggest that some Apm1 gene polymorphisms influence plasma adiponectin concentrations and lipoprotein/lipid levels. In addition, the impact of these polymorphisms is modulated by the presence of visceral obesity.


http://www.jlr.org/cgi/content/abstract/45/8/1519

Histone modification is emerging as a major regulatory mechanism for modulating gene expression by altering the accessibility of transcription factors to DNA. This study unravels the relationship between histone H3 modifications and LDL receptor induction, focusing also on routes by which phosphorylation is mediated in human hepatoma HepG2 cells. We show that while histone H3 is constitutively acetylated at LDL receptor chromatin, 12-O -tetradecanoylphorbol-13-acetate (TPA) causes rapid hyperphosphorylation of histone H3 on serine 10 (histone H3-Ser10), despite global reduction in its phosphorylation levels. Ser10 hyperphosphorylation precedes LDL receptor induction and is independent of the p42/44MAPK, p38MAPK, pp90RSK, or MSK-1 cascade. Interestingly, inhibition of protein kinase C (PKC) blocks Ser10 hyperphosphorylation and also compromises LDL receptor induction by TPA. Consistent with its role, recombinant purified PKC phosphorylate purified histone H3-Ser10. Collectively, our findings highlight a novel role for PKC in regulating histone H3-Ser10 phosphorylation and suggest that histone modification provides numerous regulatory opportunities to set the overall range of control attainable for LDL receptor gene induction.


http://www.jlr.org/cgi/content/abstract/43/3/383

Estrogen replacement therapy in women decreases hepatic lipase (HL) activity, which may account for the associated increase in HDL cholesterol. To investigate whether estrogen decreases HL transcription, transient cotransfection assays with HL promoter and estrogen receptor-(alpha) (ER(alpha)) expression constructs were performed in HepG2 cells. 17(beta)-estradiol (E2) decreased transcription driven by the -1557/+41 human HL promoter by up to 50% at 10-7 M. Mutation of ER(alpha) by deletion of its transactivation domains or ligand-binding domain eliminated E2-induced repression of the promoter, whereas deletion of the DNA-binding domain of ER(alpha) resulted in a 7-fold activation by E2. The E2-induced repression was maintained after mutation of a potential estrogen-response element in the promoter. The region of estrogen responsiveness was localized to -1557/-1175 of the HL promoter by deletion analysis. Mutation of an AP-1 site at -1493 resulted in a partial loss of E2-induced repression, similar to that caused by deletion of nucleotides -1557 to -1366. Gel shift assays with nuclear extracts from E2-treated HepG2 cells stably expressing ER(alpha) demonstrated an increase in binding to an


http://www.jlr.org/cgi/content/abstract/43/8/1303

To define the developmental expression of microsomal triglyceride transfer protein (MTP) large subunit mRNA and protein, samples of small intestine and liver were collected from 40-day gestation fetal, 2-day-old newborn, 3-week-old suckling, and 2-month-old weanling swine. In fetal animals, MTP mRNA expression was high in intestine and liver. Postnatally, jejunal expression paralleled the intake of a high-fat breast milk diet and declined after weaning. Ileal expression was comparable with that of jejunum in 2-day-old animals, but declined to low levels afterward. Hepatic expression declined postnatally and remained low. MTP protein expression generally paralleled mRNA expression, except in fetal intestine in which no 97 kDa protein was detected. In 2-day-old piglets, a high-triacylglycerol diet increased jejunal and ileal MTP mRNA levels, as compared to a low-triacylglycerol diet. To test the roles of glucocorticoids and fatty acids in MTP regulation, a newborn swine enterocyte cell line (IPEC-1) was used. Except at day 2 of differentiation, dexamethasone did not influence MTP expression. Fatty acids either up-regulated or down-regulated MTP expression, depending on the specific fatty acid and duration of exposure. Although programmed genetic cues regulate MTP expression during development, clearly the amount and fatty acid composition of dietary lipid also play regulatory roles.


http://www.jlr.org/cgi/content/abstract/44/12/2311

Fatty acid delta-6-desaturase (FADS2) is the rate-limiting enzyme in mammalian synthesis of long-chain polyunsaturated fatty acids. We investigated the molecular mechanism of FADS2 deficiency in skin fibroblasts from a patient deficient in this enzyme. Expression analyses demonstrated an 80% to 90% decrease in the steady-state level of FADS2 mRNA in patient-derived cells compared with normal controls that was consistent with previous metabolic biochemical studies. In vitro transcription assays indicated an 80% decrease in the rate of transcriptional initiation in patient-derived cells, thus implicating transcriptional regulation as the mechanism for the decreased transcript levels. Sequence analysis of the 5' end of the gene revealed the insertion of a thymidine between positions -941 and -942 upstream of the translation start site in patient-derived cells compared with normal cells and published sequences. Promoter-reporter assays demonstrated a 6-fold decrease in promoter activity in the polymorphic variant FADS2 regulatory region compared with the normal gene, confirming the functional relevance of the insertion mutation to the decreased expression of the gene in the patient-derived cells. These findings indicate that fatty acid delta-6-desaturase deficiency and decreased FADS2 transcription are caused by a nucleotide insertion in the transcriptional regulatory region of the human FADS2 gene.

http://www.jlr.org/cgi/content/abstract/44/10/1902

The objective of the present study was to examine the impact of the T111I missense mutation in exon 3 of the endothelial lipase (EL) gene on HDL and its potential interaction effect with dietary fat. The study sample included 281 women and 216 men aged between 17 and 76 years from the Quebec Family Study. Plasma HDL3-C levels of I111I homozygote women were higher compared with those of women carrying the wild-type allele (P = 0.03). These differences were not attenuated when adjusted for levels of obesity and were not observed among men. Dietary PUFA interacted with the T111I mutation to modulate apolipoprotein A-I (apoA-I) and HDL3-C levels among women. Specifically, a diet rich in PUFA was associated with increased apoA-I levels among women carriers of the I111 allele and with decreased apoA-I among women homozygotes for the wild-type allele (P = 0.002). A similar interaction was observed with plasma HDL3-C levels (P = 0.003). These interactions were not observed among men. In conclusion, the EL T111I mutation appears to have a modest effect on plasma HDL levels. The gene-diet interaction among women, however, suggests that the T111I missense mutation may confer protection against the lowering effect of a high dietary PUFA intake on plasma apoA-I and HDL3-C levels.

J. Med. Genet. (26)


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Background: Platelet derived growth factor receptor (alpha) (PDGFR(alpha)) expression is typical for a variety of brain tumours, while in normal adult brain PDGFR(alpha) expression is limited to a small number of neural progenitor cells. The molecular mechanisms responsible for the PDGFR(alpha) expression in tumours are not known, but in the absence of amplification, changes in transcriptional regulation might be an important factor in this process. Methods and results: We have investigated the link between single nucleotide polymorphisms (SNPs) within the PDGFR(alpha) gene promoter and the occurrence of brain tumours (medulloblastomas, supratentorial primitive neuroectodermal tumours (PNETs), ependymal tumours, astrocytomas, oligodendrogliomas, and mixed gliomas). These SNPs give rise to five different promoter haplotypes named H1 and H2(alpha)-delta. It is apparent from the haplotype frequency distribution that both PNET (10-fold) and ependymoma (6.5-fold) patient groups display a significant over-representation of the H2(delta) haplotype. The precise functional role in PDGFR(alpha) gene transcription for the H2(delta) haplotype is not known yet, but we can show that the H2(delta) haplotype specifically disrupts binding of the transcription factor ZNF148 as compared to the other promoter haplotypes. Conclusions: The specific over-representation of the H2(delta) haplotype in both patients with PNETs and ependymomas suggests a functional role for the ZNF148/PDGFR(alpha) pathway in the pathogenesis of these tumours.

Background: Mutations in BRAF have recently been identified in a significant percentage of primary and metastatic cutaneous malignant melanomas. As ultraviolet (UV) exposure may play a role in the development of cutaneous melanoma lesions with BRAF mutations, BRAF mutation frequency in melanomas arising in sites protected from sun exposure may be lower than those from sun-exposed areas. Thus, we determined the BRAF mutation frequency in a panel of 13 mucosal melanomas and compared those data with data from all currently published series of cutaneous melanomas. Methods: BRAF exon 15 DNA from 13 archival primary mucosal melanomas (eight vulvar, four anorectal, and one laryngeal) was sequenced using intron-based primers. As archival DNA occasionally produces poor-quality template, results were confirmed with a TspRI restriction fragment length polymorphism (RFLP) that distinguishes wild-type BRAF from the common mutant form V599E. A binomial test was used to compare the mutation frequency in the mucosal melanomas with the published mutation frequency in cutaneous melanomas. Results: None of the 13 mucosal melanomas in this series had an exon 15 BRAF mutation, as compared to 54/165 (33%) primary cutaneous melanomas with BRAF mutations in a compilation of all current published studies (p = 0.006). Discussion: These data suggest that UV exposure, plays a role in the genesis of BRAF mutations in cutaneous melanoma, despite the absence of the characteristic C>T or CC>TT mutation signature associated with UV exposure, and suggests mechanisms other than pyrimidine dimer formation are important in UV-induced mutagenesis.

http://jmg.bmjjournals.com/cgi/content/abstract/42/1/58

Objective: To clarify the genotype-phenotype correlation and elucidate the role of digenic inheritance in cystinuria. Methods: 164 probands from the International Cystinuria Consortium were screened for mutations in SLC3A1 (type A) and SLC7A9 (type B) and classified on the basis of urine excretion of cystine and dibasic amino acids by obligate heterozygotes into 37 type I (silent heterozygotes), 46 type non-I (hyperexcretor heterozygotes), 14 mixed, and 67 untyped probands. Results: Mutations were identified in 97% of the probands, representing 282 alleles (86.8%). Forty new mutations were identified: 24 in SLC3A1 and 16 in SLC7A9. Type A heterozygotes showed phenotype I, but mutation DupE5-E9 showed phenotype non-I in some heterozygotes. Type B heterozygotes showed phenotype non-I, with the exception of 10 type B mutations which showed phenotype I in some heterozygotes. Thus most type I probands carried type A mutations and all type non-I probands carried type B mutations. Types B and A mutations contributed to mixed type, BB being the most representative genotype. Two mixed cystinuria families transmitted mutations in both genes: double compound heterozygotes (type AB) had greater aminoaciduria than single heterozygotes in their family. Conclusions: Digenic inheritance is an exception (two of 164 families), with a limited contribution to the aminoaciduria values (partial phenotype) in cystinuria. Further mutational analysis could focus on one of the two genes (SLC3A1 preferentially for type I and SLC7A9 for type non-I probands), while for mixed probands analysis of both genes might be required, with priority given to SLC7A9.


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http://jmg.bmjjournals.com/cgi/content/abstract/40/9/657

Background: Hyperparathyroidism is a common endocrinopathy characterised by the formation of
parathyroid tumours. In this study, we determine the role of the recently identified gene, HRPT2, in parathyroid tumorigenesis. Methods: Mutation analysis of HRPT2 was undertaken in 60 parathyroid tumours: five HPT-JT, three FIHP, three MEN 1, one MEN 2A, 25 sporadic adenomas, 17 hyperplastic glands, two lithium associated tumours, and four sporadic carcinomas. Loss of heterozygosity at 1q24-32 was performed on a subset of these tumours. Results: HRPT2 somatic mutations were detected in four of four sporadic parathyroid carcinoma samples, and germline mutations were found in five of five HPT-JT parathyroid tumours (two families) and two parathyroid tumours from one FIHP family. One HPT-JT tumour with germline mutation also harboured a somatic mutation. In total, seven novel and one previously reported mutation were identified. "Two-hits" (double mutations or one mutation and loss of heterozygosity at 1q24-32) affecting HRPT2 were found in two sporadic carcinomas, two HPT-JT-related and two FIHP related tumours. Conclusions: The results in this study support the role of HRPT2 as a tumour suppressor gene in sporadic parathyroid carcinoma, and provide further evidence for HRPT2 as the causative gene in HPT-JT, and a subset of FIHP. In light of the strong association between mutations of HRPT2 and sporadic parathyroid carcinoma demonstrated in this study, it is hypothesised that HRPT2 mutation is an early event that may lead to parathyroid malignancy and suggest intragenic mutation of HRPT2 as a marker of malignant potential in both familial and sporadic parathyroid tumours.


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http://jmg.bmjjournals.com/cgi/content/abstract/40/6/399

MSX1 has been proposed as a gene in which mutations may contribute to non-syndromic forms of cleft lip and/or cleft palate. Support for this comes from human linkage and linkage disequilibrium studies, chromosomal deletions resulting in haploinsufficiency, a large family with a stop codon mutation that includes clefting as a phenotype, and the Msx1 phenotype in a knockout mouse. This report describes a population based scan for mutations encompassing the sense and antisense transcribed sequence of MSX1 (two exons, one intron). We compare the completed genomic sequence of MSX1 to the mouse Msx1 sequence to identify non-coding
homology regions, and sequence highly conserved elements. The samples studied were drawn from a panethnic collection including people of European, Asian, and native South American ancestry. The gene was sequenced in 917 people and potentially aetiological mutations were identified in 16. These included missense mutations in conserved amino acids and point mutations in conserved regions not identified in any of 500 controls sequenced. Five different missense mutations in seven unrelated subjects with clefting are described. Evolutionary sequence comparisons of all known Msx1 orthologues placed the amino acid substitutions in context. Four rare mutations were found in non-coding regions that are highly conserved and disrupt probable regulatory regions. In addition, a panel of 18 population specific polymorphic variants were identified that will be useful in future haplotype analyses of MSX1. MSX1 mutations are found in 2% of cases of clefting and should be considered for genetic counselling implications, particularly in those families in which autosomal dominant inheritance patterns or dental anomalies appear to be cosegregating with the clefting phenotype.


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Autosomal recessive distal renal tubular acidosis (rdRTA) is characterised by severe hyperchloraemic metabolic acidosis in childhood, hypokalaemia, decreased urinary calcium solubility, and impaired bone physiology and growth. Two types of rdRTA have been differentiated by the presence or absence of sensorineural hearing loss, but appear otherwise clinically similar. Recently, we identified mutations in genes encoding two different subunits of the renal (alpha)-intercalated cell's apical H+-ATPase that cause rdRTA. Defects in the B1 subunit gene ATP6V1B1, and the a4 subunit gene ATP6V0A4, cause rdRTA with deafness and with preserved hearing, respectively. We have investigated 26 new rdRTA kindreds, of which 23 are consanguineous. Linkage analysis of seven novel SNPs and five polymorphic markers in, and tightly linked to, ATP6V1B1 and ATP6V0A4 suggested that four families do not link to either locus, providing strong evidence for additional genetic heterogeneity. In ATP6V1B1, one novel and five previously reported mutations were found in 10 kindreds. In 12 ATP6V0A4 kindreds, seven of 10 mutations were novel. A further nine novel ATP6V0A4 mutations were found in "sporadic" cases. The previously reported association between ATP6V1B1 defects and severe hearing loss in childhood was maintained. However, several patients with ATP6V0A4 mutations have developed hearing loss, usually in young adulthood. We show here that ATP6V0A4 is expressed within the human inner ear. These findings provide further evidence for genetic heterogeneity in rdRTA, extend the spectrum of disease causing mutations in ATP6V1B1 and ATP6V0A4, and show ATP6V0A4 expression within the cochlea for the first time.

fine mapping defined a \([-]\)1.5 Mb critical region between microsatellite marker D21S1897 and the telomere of the long arm. Conclusions: CFEOM/U maps to a 1.5 Mb region at chromosome 21qter. Future identification of the disease causing gene may provide insights into the development of the extraocular muscles and brain stem \(\alpha\) motor neurones, as well as anteroposterior limb development.


http://jmg.bmjournals.com/cgi/content/abstract/41/3/155

Familial hyperparathyroidism is not uncommon in clinical endocrine practice. It encompasses a spectrum of disorders including multiple endocrine neoplasia types 1 (MEN1) and 2A, hyperparathyroidism-jaw tumour syndrome (HPT-JT), familial hypocalciuric hypercalcaemia (FHH), and familial isolated hyperparathyroidism (FIHP). Distinguishing among the five syndromes is often difficult but has profound implications for the management of patient and family. The availability of specific genetic testing for four of the syndromes has improved diagnostic accuracy and simplified family monitoring in many cases but its current cost and limited accessibility require rationalisation of its use. No gene has yet been associated exclusively with FIHP. FIHP phenotypes have been associated with mutant MEN1 and calcium-sensing receptor (CASR) genotypes and, very recently, with mutation in the newly identified HRPT2 gene. The relative proportions of these are not yet clear. We report results of MEN1, CASR, and HRPT2 genotyping of 22 unrelated subjects with FIHP phenotypes. We found 5 (23%) with MEN1 mutations, four (18%) with CASR mutations, and none with an HRPT2 mutation. All those with mutations had multiglandular hyperparathyroidism. Of the subjects with CASR mutations, none were of the typical FHH phenotype. These findings strongly favour a recommendation for MEN1 and CASR genotyping of patients with multiglandular FIHP, irrespective of urinary calcium excretion. However, it appears that HRPT2 genotyping should be reserved for cases in which other features of the HPT-JT phenotype have occurred in the kindred. Also apparent is the need for further investigation to identify additional genes associated with FIHP.


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http://jmm.sgmjournals.org/cgi/content/abstract/52/11/1015

The aim of the present study was to investigate the relative contribution of enteropathogenic Escherichia coli (EPEC) as a cause of infectious diarrhoea in Norwegian children. Data from
faecal specimens from children <2 years old with diarrhoea during the year 2001 were analysed. E. coli isolates with the attaching and effacing genotype (eae+) were examined for the presence of the bundle-forming pilus (bfpA) and Shiga toxin genes by PCR, and for genetic relatedness by PFGE. During the 1-year period, 598 specimens from 440 patients <2 years old were analysed. Potential enteric pathogens were identified in 124 patients (28.2 %). EPEC was the most frequently identified agent (44 patients), followed by rotavirus (41 patients), Campylobacter jejuni (17 patients) and adenovirus (17 patients). All other agents were detected in five patients or less. Only one of the eae+ E. coli isolates was classified as typical EPEC (bfpA+). Among the 43 isolates that were classified as atypical EPEC (bfpA-), eight strains belonged to EPEC serogroups, whereas the majority of strains (n = 35) were not agglutinated by EPEC antisera. None of the EPEC isolates were genetically related. This study demonstrates that atypical EPEC of non-EPEC serogroups is highly prevalent among Norwegian children with diarrhoea.


http://jmm.sgmjournals.org/cgi/content/abstract/53/11/1137

The aim of the present case control study was to investigate the prevalence of atypical enteropathogenic Escherichia coli (EPEC) and its possible role in causing diarrhoea among children < 5 years of age in Norway. Stool specimens received in the laboratory from children with suspected gastroenteritis (n = 251) were, in addition to routine testing, analysed for the presence of EPEC by PCR of the eae, bfpA and stx genes. Specimens from healthy children (n = 210) recruited from Maternal and Child Health Centres were analysed for EPEC only. EPEC isolates (eae+, stx-) were classified as typical (bfpA+) or atypical (bfpA-), and were tested for O: K serogroup. Information on duration of diarrhoea was recorded in a questionnaire and from referral forms. Atypical EPEC was diagnosed in 37 patients (14.7 %) compared to 21 (10.0 %) of the healthy controls [Odds ratio (OR) = 1.4, P = 0.3]. Only three isolates, all from patients, belonged to EPEC serogroups. One patient had typical EPEC. Twenty (22.5 %) of 89 patients with diarrhoea lasting [≥]14 days had atypical EPEC. The association between atypical EPEC and prolonged diarrhoea (OR = 2.1, P = 0.04) was caused by a high prevalence among female patients (40.6 %). In conclusion, atypical EPEC was found to be slightly more prevalent in patients than controls, without any overall significant association with diarrhoea. However, a significant association was observed with diarrhoea lasting 14 days or more, a finding that may indicate a role for atypical EPEC in prolonged disease.


http://jmm.sgmjournals.org/cgi/content/abstract/53/7/633

Candida dubliniensis is an emerging pathogen capable of causing oropharyngeal, vaginal and bloodstream infections. Although C. dubliniensis is similar to Candida albicans in several phenotypic characteristics, it differs from it with respect to epidemiology, certain virulence factors and the ability to develop resistance to fluconazole rapidly. In this study, the first seven isolations of C. dubliniensis from Kuwait are described, all originating from non-human immunodeficiency virus (HIV)-infected patients. The isolates were initially identified by the Vitek 2 yeast identification system, positive germ tube test, production of rough colonies and chlamydospores on Staib agar and by their inability to assimilate xylose, trehalose or methyl {alpha}-D-glucoside. The species identity of the isolates was subsequently confirmed by specific amplification of rDNA targeting the internally transcribed spacer 2 (ITS2), restriction endonuclease digestion of the amplified DNA
and direct DNA sequencing of the ITS2. Using the E-test method, the MICs of C. dubliniensis test isolates were in the range 0.125-0.75 \( \mu \text{g} \text{ ml}^{-1} \) for fluconazole, 0.002-0.75 \( \mu \text{g} \text{ ml}^{-1} \) for itraconazole, 0.006-0.125 \( \mu \text{g} \text{ ml}^{-1} \) for ketoconazole, 0.002-0.5 \( \mu \text{g} \text{ ml}^{-1} \) for amphotericin B and 0.002-0.016 \( \mu \text{g} \text{ ml}^{-1} \) for voriconazole. Two of the isolates were resistant to 5-flucytosine (>32 \( \mu \text{g} \text{ ml}^{-1} \)), but none against fluconazole. The study reinforces the current view that C. dubliniensis has a much wider geographical and epidemiological distribution.


http://jmm.sgmjournals.org/cgi/content/abstract/52/9/765

Helicobacter species are fastidious bacterial pathogens that are difficult to culture by standard methods. A PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique for detection and identification of different Helicobacter species was developed and evaluated. The method involves PCR detection of Helicobacter DNA by genus-specific primers that target 16S rDNA and subsequent differentiation of Helicobacter PCR products by use of DGGE. Strains are identified by comparing mobilities of unknown samples to those determined for reference strains; sequence analysis can also be performed on purified amplicons. Sixteen DGGE profiles were derived from 44 type and reference strains of 20 Helicobacter species, indicating the potential of this approach for resolving infection of a single host by multiple Helicobacter species. Some more highly related species were not differentiated whereas in highly heterogeneous species, sequence divergence was observed and more than one PCR-DGGE profile was obtained. Application of the PCR-DGGE method to DNA extracted from faeces of zoo animals revealed the presence of Helicobacter DNA in 13 of 16 samples; a correlation was seen between the mobility of PCR products in DGGE analysis and DNA sequencing. In combination, this indicated that zoo animals are colonized by a wide range of different Helicobacter species; seven animals appeared to be colonized by multiple Helicobacter species. By this approach, presumptive identifications were made of Helicobacter bilis and Helicobacter hepaticus in a Nile crocodile, Helicobacter cinaedi in a baboon and a red panda, and Helicobacter felis in a wolf and a Taiwan beauty snake. All of these PCR products (~400 bp) showed 100 % sequence similarity to 16S rDNA sequences of the mentioned species. These results demonstrate the potential of PCR-DGGE-based analysis for identification of Helicobacter species in complex ecosystems, such as the gastrointestinal tract, and could contribute to a better understanding of the ecology of helicobacters and other pathogens with a complex aetiology.


http://jmm.sgmjournals.org/cgi/content/abstract/52/9/759

Laboratory detection of Pseudomonas spp., in particular Pseudomonas aeruginosa, remains an important assay in the management of patients with cystic fibrosis (CF). As the groES and groEL genes of P. aeruginosa have now been cloned and their nucleotide sequences determined, the aim of this study was to develop a novel PCR assay for the detection of Pseudomonas spp. from patients with CF by employing conserved primer regions of the groE heat-shock protein domain gene. A PCR assay was designed that targeted a 536 bp region of the groE gene to detect Pseudomonas spp. PCR amplification of genomic DNA from extracted organisms generated an amplicon of the expected size (approx. 536 bp) for all P. aeruginosa (n = 60), Pseudomonas putida, Pseudomonas fluorescens and Pseudomonas stutzeri isolates examined, but did not
produce a positive amplicon for several other genera and species that are commonly isolated from the sputum of CF patients. RFLP analysis of the amplicons of all P. aeruginosa isolates demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp; direct sequencing of the amplicons demonstrated the presence of a single sequence type, indicating the highly conserved nature of this region. In addition, the assay successfully produced a positive signal from primary non-selective plates of three known P. aeruginosa culture-positive CF patients, but was unable to generate a signal in a further six CF patients who had no history of infection with P. aeruginosa or other Pseudomonas spp. This assay is recommended to detect the presence of Pseudomonas spp., including P. aeruginosa, from primary culture plates that originate from laboratory analysis of CF patients' sputum, particularly at review, in those patients with no previous history of Pseudomonas infection or those who appear to be transiently colonized by this organism. Employment of such molecular methodologies, in conjunction with routine clinical sputum cultures, may provide improved information on the microbial status of CF patients, which will aid clinicians in both optimum patient management in terms of antibiotic regimes and CF centre infection-control practices.


http://jmm.sgmjournals.org/cgi/content/abstract/53/6/519

This study reports on practical laboratory aspects of pertussis diagnosis. PCR assays were applied to respiratory specimens obtained during a large study of infants (less than 5 months old) admitted to paediatric intensive care units (n = 122), children (less than 15 years old) admitted to paediatric wards (n = 16) and their household contacts (n = 320). Estimation of antibodies to pertussis toxin and culture for Bordetella pertussis were attempted on specimens from the same patients, where available, and the overall utility of the diagnostic PCR assays was assessed by comparison to these results. A PCR assay for the human mitochondrial cytochrome oxidase (HMCO) gene was used for quality control of the extracted samples and an internal process control (IPC) was included in each sample to test for PCR inhibition. Four of 458 samples were considered unsuitable (three HMCO negative, one IPC negative) and excluded from further analyses. Positive PCR results were considered valid if they were either (i) positive for both of two B. pertussis gene targets (pertussis toxin S1 promoter and the insertion element IS481), i.e. consensus PCR positive, or (ii) repeatably positive in only one assay. Using these criteria, 52 of 454 (11.5 %) samples were considered as PCR positive for B. pertussis. Six of 356 samples were culture-positive for B. pertussis, 1/88 infants, 3/14 children and 2/254 contacts, giving an overall isolation rate of 1.7 %. Using these data, PCR gave an almost fivefold increase in diagnostic yield compared with culture (McNemar's test; P < 0.0001). Sera from 9/111 infants, 5/10 children and 14/210 contacts were positive. Serology and PCR results showed a high level of agreement (113/121) for infants and children. PCR demonstrated a significant improvement in diagnostic yield over culture. Serological testing also resulted in a significant increase in diagnostic yield compared to culture alone. PCR is a useful technique, but validity of results must be assured by careful control. Rapid diagnosis of B. pertussis infection particularly in infants by PCR, together with serological assays, can enhance surveillance systems for pertussis in all age groups.


http://jmm.sgmjournals.org/cgi/content/abstract/51/2/178

Streptococcus intermedius belongs to the anginosus group of streptococci (AGS) and is associated with endogenous infections leading to abscesses in the oral cavity and at deep-seated
sites, such as the brain and liver. Two other species, S. anginosus and S. constellatus, and some presently unnamed taxa, are also classified as AGS. Recently, S. constellatus subsp. pharyngis, a new subspecies with biochemical characteristics similar to S. intermedius, was described with the potential for causing confusion when trying to identify isolates of these two species routinely with commercial identification kits, such as Rapid ID32 Strep and Fluo-Card Milleri. To correctly identify S. intermedius, this study attempted to develop an accurate PCR identification system with the ily gene as a species marker. This approach relies on amplification of an 819-bp fragment of the ily gene and its 3'-flanking region and is shown here to be specific for S. intermedius strains among all other streptococcal species. Moreover, this PCR system was applicable in direct rapid PCR with whole bacterial cells and TaKaRa Z-TaqTM (TaKaRa), a highly efficient DNA polymerase, as the template and DNA amplification enzyme, respectively.


http://jmm.sgmjournals.org/cgi/content/abstract/54/1/23

A real-time LightCycler PCR (LC-PCR) with hybridization probes for detection of Mycoplasma genitalium in endocervical and first void urine specimens was developed and compared to a conventional PCR. The primers for both assays were identical and designed to amplify a 427 bp fragment of the 16S rRNA gene of M. genitalium. The LC-PCR assay had a detection limit of < 5 bacterial genomes per reaction when dilutions of genomic DNA from a type strain of M. genitalium were tested. First void urine from 398 men and first void urine and endocervical specimens from 301 women attending an STD clinic were analysed by LC-PCR and by the conventional PCR. Using the conventional PCR as reference, the LC-PCR had a specificity of 99.7 % and a sensitivity of 72.2 % for the detection of M. genitalium in first void urine samples from men. There was no significant difference in the performance of the LC-PCR assay compared to the conventional PCR when endocervical swabs were considered (58 and 65 %, respectively) or with a set of endocervical swab/urine specimens for which the LC-PCR assay detected 73 % of the infections (specificity = 98.6 % and sensitivity = 68.2 %) while the conventional PCR detected 85 % of the infections. With female urine specimens there was a significant difference between the two assays (38 and 73 %, respectively; P = 0.01 McNemar's test). This illustrates the need to analyse both endocervical and urine specimens, because M. genitalium DNA was detected in only one of the two specimens in a great number of the M. genitalium-infected women. The lower sensitivity of the LC-PCR assay was probably caused by a combination of inhibition and limitations regarding the amount of template DNA. The LC-PCR assay was easy to perform and the simultaneous amplification and detection eliminated the need for further handling of PCR products. With improvement in sample preparation methods and increased volumes of the template DNA, the LC-PCR assay could be a useful routine diagnostic method.


http://jmm.sgmjournals.org/cgi/content/abstract/51/4/305

The accuracy of the urea breath test (UBT) and histological grading for estimation of the density of Helicobacter pylori in gastric mucosa is not known. Real-time (TaqMan) PCR was used to estimate the total number of H. pylori genomes in biopsy samples. These values were compared with those obtained by the UBT and the histological grade obtained by the Sydney system. The UBT and endoscopy with antral and corporal biopsies were performed in 88 consecutive
untreated patients with dyspepsia. Bacterial culture and the rapid urease test were done with fresh biopsy materials. TaqMan PCR and histological examination were done on serial paraffin sections of the biopsy samples. Of the five methods tested, TaqMan PCR had the highest sensitivity and specificity (both 100%) in the diagnosis of H. pylori infection. The mean density of H. pylori genomes for pairs of biopsy samples from individual patients was compared with the individual values obtained by the UBT; correlation between the results was significant. The density of H. pylori genomes was higher in histological grades 1, 2 and 3 than in grade 0, without significant differences between adjacent grades from 1 to 3. These results suggest that the severity of H. pylori infection of the stomach can be estimated by the UBT and that histopathologists might state whether the organism is present or absent, rather than making a quantitative statement as recommended in the Sydney system.


http://jmm.sgmjournals.org/cgi/content/abstract/53/9/927

The purpose of this study was to develop a simple procedure for cell lysis and DNA extraction for direct detection of Mycobacterium ulcerans in aquatic insects, gills and intestinal contents of fish, molluscs and human tissue samples using a nested PCR method specific for the insertion sequence IS2404. The simultaneous action of sodium N-lauroyl sarcosine, guanidinium isothiocyanate, chloroform and Tris-saturated phenol on mycobacteria, followed by a DNA purification method using mini-columns fitted with silica-cellulose membranes was successfully employed to extract DNA from cultured bacteria, environmental and human tissue samples. All specimens were collected from Buruli ulcer endemic regions. M. ulcerans DNA was detected in 11 of 57 aquatic insects, one of six molluscs and three of 15 fish, supporting the hypothesis that the fauna of major Buruli ulcer endemic foci in swampy terrain of tropical and subtropical regions can be a source of M. ulcerans infection.


http://jmm.sgmjournals.org/cgi/content/abstract/54/4/369

Early and rapid detection of the causative organism is necessary in tuberculosis, particularly tuberculous meningitis, as the disease affects mainly children and if untreated or improperly treated can cause severe central nervous system disorders and can often be fatal. An in-house-developed PCR technique was developed for the detection of Mycobacterium tuberculosis DNA, in which the target for amplification was a 340 bp nucleotide sequence located within the 38 kDa protein gene. The test can detect as small an amount of DNA as 10 fg, which is equivalent to two to three organisms, and is highly specific. Amplified product was detected by ethidium bromide staining after electrophoresis and Southern hybridization. Evaluation of test sensitivity and specificity was carried out using acid-fast bacilli-positive sputum samples from patients with pulmonary tuberculosis and an equal number of non-tuberculosis patient samples as negative controls. In a double-masked study 30 cerebrospinal fluid samples from tuberculous meningitis patients and 30 samples from non-tuberculous meningitis patients were investigated. Out of the 30 samples 22 were positive by ethidium bromide-stained gel electrophoresis and 27 gave positive results by Southern hybridization. All of the 30 control samples showed negative results. The sensitivity of this PCR was 90 % and specificity, 100 %.

http://jmm.sgmjournals.org/cgi/content/abstract/51/1/20

The immunogenicity and protective efficacy of a DNA vaccine encoding the GroEL heat-shock gene from Brucella abortus was tested in BALB/c mice immunised by intramuscular (i.m.) needle injection or epidermally by gene gun. The Brucella GroEL gene was amplified by PCR and cloned into two different mammalian expression vectors pCMV-link and pCMV-tPA. The D17 cell line was transfected with both constructs and GroEL transcripts were detected by Northern blot. To determine the level of protein synthesised, transfected cell lysates were then submitted to Western blot. The non-secreted form of the recombinant GroEL produced by the pCMV-link construct was detected in much greater amount than the secreted form of the protein produced by the pCMV-tPA construct. After immunisation, a strong anti-GroEL IgG response was detected in mice vaccinated by i.m. injection or gene gun only when the pCMV-link/GroEL plasmid was used. Regarding the pattern of immune response induced, i.m. needle injection raised a predominantly Th1 response with mostly IgG2a-specific anti-GroEL and high levels of IFN-{gamma} produced by splenic T cells. Gene gun immunisation induced a Th0 type of immune response in mice characterised by a high IgG1/IgG2a ratio, and IL-4 and interferon (IFN)-{gamma} production. Even though a distinct pattern of immune response was generated depending upon the immunisation route used, neither method engendered a significant level of protection with the GroEL DNA vaccine.


http://jmm.sgmjournals.org/cgi/content/abstract/52/11/999

Mutations in the katG locus of catalase peroxidase in Mycobacterium tuberculosis (MTB) account for major isoniazid (INH) resistance. In the South China region, a collection of 906 respiratory specimens and 142 MTB isolates was used to evaluate the sensitivity and specificity of a PCR-RFLP method for the detection of INH resistance-associated mutations. Except for four catalase-negative MTB isolates, katG PCR for a 620-bp amplicon was successful for all purified MTB isolates. For respiratory specimens, diagnostic sensitivity and specificity of katG PCR was 85 and 100 %. Subsequent RFLP of the katG amplicons by MspI digestion identified that 51 % of INH-resistant MTB were associated with the Thr315 phenotype, and that codon 463 was a polymorphic site with no linkage to INH resistance. The Arg463 wild-type MTB isolates predominant in the Western world were replaced by isolates carrying Leu463 in the South China region. RFLP patterns of katG amplicons from respiratory specimens were identical to those of the corresponding MTB cultured colonies. This method has potential application for rapid diagnosis of INH resistance due to katG Ser315Thr mutation.


http://jmm.sgmjournals.org/cgi/content/abstract/51/2/117
Diagnosis of dermatophytosis employing conventional laboratory procedures has been complicated by the slow growth and varied morphological features shown by dermatophytes. After analysis of the nucleotide base sequences of a 1.2-kb fragment amplified from a dermatophyte fungus Trichophyton rubrum by arbitrarily primed PCR with random primer OPD18, a pair of primers (TR1F and TR1R) was designed and evaluated for specific identification of T. rubrum. The sensitivity of the primers TR1F and TR1R was high, as a specific PCR band of c. 600 bp was detected from as little as 7 pg of T. rubrum DNA. By examining 92 dermatophyte strains and clinical isolates, it was found that this pair of primers reacted in PCR with T. rubrum, T. soudanense and T. gourvilii through formation of the specific fragment of 600 bp, but not with any other of the dermatophyte species or varieties, fungi, yeasts or bacteria tested. As T. rubrum is one of the most frequently isolated dermatophyte fungi, and T. soudanense and T. gourvilii are relatively uncommon in many parts of the world, these primers can be used for rapid, sensitive and specific identification and differentiation of T. rubrum from other fungi and micro-organisms.


http://jmm.sgmjournals.org/cgi/content/abstract/52/11/955

Variants of the p55 gene in rat-derived Pneumocystis carinii have been identified and its counterpart in mouse-derived P. carinii f. sp. muris has been cloned. By PCR amplification of P. carinii genomic DNA, five variants were identified that differed from each other in size and sequence, primarily in the number and size of encoded amino acid repeats. For P. carinii f. sp. muris, a single PCR fragment (471 bp) was obtained, which contained an incomplete ORF encoding a 157 aa protein that was most similar to a p55 variant in P. carinii, with nucleotide and amino acid sequence identity of 79 and 68 %, respectively. Southern blot analysis revealed the presence of more than one copy of the p55 gene in both Pneumocystis species. Thus, like other Pneumocystis antigens, p55 exhibits polymorphism that could potentially benefit the organism in host interactions.


http://jmm.sgmjournals.org/cgi/content/abstract/51/1/34

The production of toxins A and B by Clostridium difficile was greatly enhanced under biotin-limited conditions, in which a 140-kDa protein was expressed strongly. Gene cloning revealed that this protein was a homologue of formylglycinamidine ribonucleotide synthetase (FGAM synthetase, EC 6.3.5.3), which is known as PurL in Escherichia coli and catalyses the fourth step of the de novo purine biosynthesis pathway. This enzyme consisted of a single polypeptide, although FGAM synthetases of gram-positive bacteria usually consist of two subunits. Inhibition of the enzymic activity of C. difficile PurL by O-diazoacetyl-L-serine (azaserine) resulted in enhanced toxin B production even in biotin-sufficient conditions. In contrast, blockade of the preceding step of the PurL catalysing step by sulfamethoxazole inhibited toxin B production almost completely. These results suggest that accumulation of formylglycinamidine ribonucleotide (FGAR), a substrate of FGAM synthetase, enhances toxin production by C. difficile and depletion of FGAR reduces toxin production.

Paciorek, J. (2002). "Virulence properties of Escherichia coli faecal strains isolated in Poland from healthy..."
Four hundred and twenty-seven Escherichia coli isolates from 427 cases of infantile diarrhoea in Poland, belonging to serogroups O18, O26, O44, O86, O126 and O127 and 150 E. coli isolates from 52 healthy children were examined for selected virulence properties. The presence of the plasmid pAA, a plasmid encoding enterohaemolysin, the genes encoding intimin (eae), bundle-forming pili (bfp), Shiga toxins I and II (stxl, stxII) and cytotoxic necrotising factor types 1 and 2 (cnf1, cnf2) was investigated by PCR. Adhesion to HEp-2 cell monolayers was also tested and selected strains were investigated for the presence of P-fimbriae and haemolytic activity. Typical enteropathogenic E. coli isolates (typical EPEC, strains possessing eae and bfp, but not stx) were not found. The particular classes of E. coli among 427 isolates from ill children were: atypical EPEC (eae+ bfp, stx-), 21.3%; Shiga toxin-producing E. coli (STEC), 0.7%; diffusely adherent E. coli (DAEC), 4%; enteroaggregative E. coli (EAEC), 16.9%; necrotoxic E. coli type 1 (NTEC1), 0.2%; and cell-detaching E. coli (CDEC), 29%. With the exception of STEC, all the above classes of E. coli were found among the isolates from healthy children which comprised: atypical EPEC 8.0%, DAEC 6.7%, EAEC 17.3%, NTEC1 14.0% and CDEC 40.0%. Cell detachment (CD) was significantly associated with 3-h haemolytic activity. There was also strong correlation between haemolytic activity (Hly) and the presence of P-fimbriae. No correlation was found between the presence of the cnf1 gene and CD, Hly or P-fimbriae.


Genes for the outer-surface protein C (OspC) from three north European human isolates of Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii were cloned and sequenced. Polyhistidine-tagged recombinant OspC (rOspC) proteins were produced in Escherichia coli and used, after biotinylation, as antigens on streptavidin-coated plates in enzyme-linked immunosorbent assays (ELISA). In IgM ELISA, 30% (5/17) and 35% (6/17) of patients with erythema migrans (EM) in the acute or convalescent phase, respectively, reacted with one to three rOspCs. Of the patients, 53% (8/15) with neuroborreliosis (NB) and 53% (8/15) with Lyme arthritis (LA) had IgM antibodies to OspC. The immunoreactivity was stronger against rOspC from B. afzelii and B. garinii than against rOspC from B. burgdorferi sensu stricto. In early Lyme borreliosis (LB), rOspC and flagella performed equally well in detecting IgM antibodies. Cross-reactive antibodies to rOspC were observed in serum samples from patients with rheumatoid factor positivity and with syphilis or Epstein-Barr virus (EBV) infection. In IgM ELISA, thiocyanate in the serum dilution buffer reduced EBV-associated non-specific positive reactions. Of the patient sera examined in IgG ELISA, 30% (5/17) with EM in the acute phase, 35% (6/17) with EM in the convalescent phase, 33% (5/15) with NB and 60% (9/15) with LA were positive. Because of the heterogeneity of OspC, a polyvalent antigen with several OspC variants from at least B. afzelii and B. garinii is needed to improve the sensitivity of OspC ELISA in the serodiagnosis of LB in Europe.

A cosmid DNA library had been constructed previously from 40-kb fragments of genomic DNA from a virulent invasive strain of Salmonella enterica serotype Typhimurium (TML) in an avirulent hypo-invasive Typhimurium strain (LT7). Selection of invasive clones from the library was attempted by iterative passage through a rabbit ileal organ culture. After the fourth passage, a clone, designated LT7(pHC20uu.2), was isolated. Exposure to both gut tissue and Caco-2 cells enhanced the growth, invasiveness for gut and Caco-2 cells, and flagellin expression of LT7(pHC20uu.2) although its invasiveness was less than that of strain TML. Expression of appendages (surface structures c. 60-70 nm diameter) was shown to play a role in but not to confer invasiveness, and was demonstrated in the absence of direct contact with eukaryotic cells. Exposure to gut tissue also affected the expression of several outer-membrane proteins (OMPs) in all four Salmonella strains - TML, LT7, LT7(pHC79), LT7(pHC20uu.2) - used in this work. As the genes involved in flagella, invasin and porin expression are distributed around the salmonella chromosome, it is possible that pHC20uu.2 encodes a pleiotropic regulator of genes involved in gastro-enteritic virulence and adaptation to the in-vivo gut environment. pHC20uu.2 mapped at c. centisome 25 on the salmonella chromosome close to, but distinct from, SPI-5.


http://jmm.sgmjournals.org/cgi/content/abstract/52/8/653

Using gellan gum as a substitute for agar-agar in a mycological medium and sequencing of the ITS 1 and 2 regions resulted in an accurate identification of Aspergillus fumigatus, Aspergillus nidulans, Aspergillus terreus and Aspergillus ustus within 24 h of subculture.


http://jmm.sgmjournals.org/cgi/content/abstract/51/10/851

Distinct virulence factors of Helicobacter pylori have been associated with clinical outcome of the infection; however, considerable variations have been reported from different geographic regions. Data on genotypes of African H. pylori isolates are sparse. The aim of this study was to determine the prevalence of specific genotypes of H. pylori in Nigerian patients with duodenal ulcer and non-ulcer dyspepsia. H. pylori was cultured from endoscopic biopsies obtained from 41 Nigerian patients (19 with duodenal ulcer, 22 with non-ulcer dyspepsia). The vacA alleles, cagA and iceA genotypes were determined by PCR. The vacA s1,m1 and s1,m2 genotypes were found in 26.3% and 22.7%, and in 73.7% and 72.7% of H. pylori isolates from patients with duodenal ulcer and non-ulcer dyspepsia, respectively. The iceA1 genotype was present in 94.7% and 86.4% of isolates from duodenal ulcer and non-ulcer dyspepsia patients, respectively. cagA+ infection was found predominantly (>90%) in Nigerian H. pylori isolates irrespective of the clinical diagnosis. In conclusion, vacA s1,m2, iceA1 and cagA+ are common genotypes of H. pylori isolated from Nigerian patients. As in several other developing countries there seems to be no association between these genotypes and duodenal ulcer disease.

Fifteen Bacteroides fragilis isolates from the USA, Hungary and Kuwait were examined for carbapenem resistance, for carbapenemase activity and, with the use of various PCR-based methods and nucleotide sequencing, for cfiA genes and activating insertion sequence (IS) elements. All the B. fragilis isolates were cfiA-positive, 10 of the cfiA genes being upregulated by IS elements that are already known. Of these 10, one was of a novel type (designated IS943) and two further ones (IS614B and IS614C) were suspected hybrids of IS612, IS614 and IS942. There were five cfiA-positive imipenem-resistant B. fragilis isolates with elevated imipenem MICs (minimal inhibitory concentration) that harboured no IS insertion upstream of the cfiA gene, but produced carbapenemase; these isolates might possess a novel activation mechanism. On the basis of the available phenotypic and genotypic evidence, the present data suggest that there are at least two cfiA activation mechanisms among B. fragilis isolates.


The objective of this study was to understand more of the innate immune response to Helicobacter pylori by determining the expression of human (beta)-defensin-2 (hBD-2) in various gastric mucosal tissues and MKN45 gastric cancer cells with or without H. pylori. Semi-quantitative TaqMan RT-PCR and immunohistochemistry were carried out. The antimicrobial effects of a transfected hBD-2 gene against H. pylori were also evaluated. The results showed that hBD-2 was expressed in inflamed gastric mucosal tissues with H. pylori infection, but not in the absence of H. pylori infection. Expression was also detected in gastric cancers in patients with H. pylori infection. Expression was induced in the MKN45 gastric cancer cell line by H. pylori in a manner dependent on the abundance of bacteria. hBD-2-transfected 3T3J2-1 cells secreted hBD-2 protein into the culture medium and this protein inhibited growth of H. pylori completely. The results suggest that hBD-2 may be involved in the pathophysiology of H. pylori-induced gastritis.


Mushroom worker's lung (MWL) is a hypersensitivity pneumonitis or allergic alveolitis caused by a type III IgG-mediated immunopathogenic inflammatory reaction in the host due to the inhalation of several thermophilic organisms, including Thermoactinomyces spp. It is difficult to distinguish phenotypically the eight species of this genus; therefore, this study sought to develop an improved molecular means of identifying Thermoactinomyces spp. associated with MWL by partial 16S rDNA PCR amplification and direct sequencing. Hypervariable regions within the 16S rRNA gene, which could be employed as signature sequences of the eight individual species, were identified and employed with highly conserved flanking primers to allow initial PCR amplification, before direct DNA sequencing of the 16S rDNA amplicons. A novel 24-mer 16S rDNA oligonucleotide upstream primer was designed from in silico alignments of all Thermoactinomyces spp. and was employed in combination with downstream (reverse) 16S rDNA primers. This permitted the successful identification of all four isolates associated with mushroom workers' lung. The method may be useful in the identification of Thermoactinomyces.
spp. associated with allergic alveolitis or pneumonitis associated with occupational exposure in agricultural and horticultural environments.

J. Mol. Endocrinol.  (2)


http://jme.endocrinology-journals.org/cgi/content/abstract/34/1/61

To explore the mechanisms whereby estrogen and antiestrogen (tamoxifen (TAM)) can regulate breast cancer cell growth, we investigated gene expression changes in MCF7 cells treated with 17(β)-estradiol (E2) and/or with 4-OH-TAM. The patterns of differential expression were determined by the ValiGen Gene IDentification (VGID) process, a subtractive hybridization approach combined with microarray validation screening. Their possible biologic consequences were evaluated by integrative data analysis. Over 1000 cDNA inserts were isolated and subsequently cloned, sequenced and analyzed against nucleotide and protein databases (NT/NR/EST) with BLAST software. We revealed that E2 induced differential expression of 279 known and 28 unknown sequences, whereas TAM affected the expression of 286 known and 14 unknown sequences. Integrative data analysis singled out a set of 32 differentially expressed genes apparently involved in broad cellular mechanisms. The presence of E2 modulated the expression patterns of 23 genes involved in anchors and junction remodeling; extracellular matrix (ECM) degradation; cell cycle progression, including G1/S check point and S-phase regulation; and synthesis of genotoxic metabolites. In tumor cells, these four mechanisms are associated with the acquisition of a motile and invasive phenotype. TAM partly reversed the E2-induced differential expression patterns and consequently restored most of the biologic functions deregulated by E2, except the mechanisms associated with cell cycle progression. Furthermore, we found that TAM affects the expression of nine additional genes associated with cytoskeletal remodeling, DNA repair, active estrogen receptor formation and growth factor synthesis, and mitogenic pathways. These modulatory effects of E2 and TAM upon the gene expression patterns identified here could explain some of the mechanisms associated with the acquisition of a more aggressive phenotype by breast cancer cells, such as E2-independent growth and TAM resistance.


http://jme.endocrinology-journals.org/cgi/content/abstract/34/1/107

Androgen-independent prostate cancer is a lethal form of the disease that is marked by metastasis and rapid proliferation in its final stages. As no effective therapy for this aggressive tumor currently exists, it is imperative to elucidate and target the mechanisms involved in the progression to androgen independence. Accumulating evidence indicates that aberrant activation of androgen receptor (AR) via signal transduction pathways, AR gene mutation and/or amplification, and/or coregulator alterations may contribute to the progression of prostate cancer.
In the present study, the effects of protein kinase A (PKA) signaling and its downstream factors on AR activity at the prostate-specific antigen (PSA) gene were tested. Activation of PKA by forskolin resulted in enhanced androgen-induced expression of the PSA gene, an effect that was blocked by the AR antagonist, bicalutamide. Interestingly, when either p300 or CBP was overexpressed, PKA activation was sufficient to stimulate PSA promoter-driven transcription in the absence of androgen, which was not inhibited by bicalutamide. PKA activation did not significantly alter AR protein levels but significantly increased the phosphorylated form of its downstream effector, cAMP responsive element-binding protein (CREB) in the presence of androgen. Furthermore, chromatin immunoprecipitation showed that the combination of androgen and forskolin increased phosphorylated CREB occupancy, which was accompanied by histone acetylation, at the putative cAMP responsive element located in the 5' upstream regulatory region of the PSA gene. Remarkably, mammalian two-hybrid assay indicated that p300/CBP may bridge the interaction between AR and CREB, suggesting a novel enhanceosomal cooperation. These results demonstrate an intriguing interplay between a signal transduction pathway, coactivator overexpression and AR signaling as a possible combined mechanism of progression to androgen-independent prostate cancer.

J. Mollus. Stud. (4)


http://mollus.oupjournals.org/cgi/content/abstract/68/1/17

Full-length actin-encoding sequences were PCR-amplified from genomic DNA of six planorbid species; Biomphalaria glabrata (Say; M-line strain), B. alexandrina (Ehrenberg), B. pfeifferi (Krauss), B. tenagophila (Orbigny), B. obstricta (Morelet) and Helisoma trivolvis (Say), using primers designed from a previously reported B. glabrata cytoplasmic (β) actin cDNA. The amplified sequences contained two conserved exons (126 nt and 1005 nt, respectively), separated by an intron that varied in size between snail species (ranging from 671 to 794 nt). Sequence similarities occurred between the introns of the actin genes from B. glabrata, B. alexandrina and B. pfeifferi and between those from B. tenagophila and B. obstricta, yet considerable differences were evident between these two groups and the intron derived from H. trivolvis. Analysis of exons for sequence similarities, the presence of conserved residues (deduced amino acids), and construction of gene trees indicated that these planorbid genes encode cytoplasmic (β) actins rather than muscular (α) actins. Southern blotting and hybridisation experiments suggested that B. glabrata and H. trivolvis may have multiple (up to 5) actin genes, and it can not be ruled out that actin sequences obtained from different planorbid species were derived from paralogous genes. Interestingly however, the gene trees resolved actins derived from gastropod, cephalopod and bivalve molluscs. The sequences presented add to the growing body of information on the molecular biology of planorbid snails.

The phylogenetic relationships among selected members of the family Margaritiferidae are investigated using sequence data from five molecular markers. Parsimony analyses of the data support the recognition of those nominal species for which multiple samples were included in the study (Margaritifera margaritifera, M. laevis, M. falcata and M. auricularia). Although not always strongly supported, the following relationships were consistently recovered: (Cumberlandia + Margaritifera auricularia), (M. falcata (M. marrianae + M. laevis)) and to a lesser degree (Dahurinaia dahurica + M. margaritifera). The molecular phylogeny indicates that the taxonomy of the group is in need of revision since the genus Margaritifera is not monophyletic and a new taxonomy by Smith (2001) is not supported. A complicated pattern of biogeography was suggested by the three clades of Old World + New World species. It is difficult to determine whether this pattern is a reflection of extinction and contraction of an ancient, formerly widespread margaritiferid fauna, peripheral isolation of formerly widespread taxa, fish host dispersal, or even host switching.


Objective: Chlamydia pneumoniae (C pneumoniae) is a common cause of a usually mild, community acquired pneumonia. This organism, however, can spread from the respiratory tract into other parts of the body and has been detected in up to 70% of atheromatous lesions in blood vessels. Although the exact mechanism of the C Pneumoniae contribution to the pathogenesis of atherosclerosis remains unknown, prophylactic antibiotic trials are planned for people at high risk for coronary disease. Method: In this study the authors aimed to investigate C pneumoniae DNA content in the cerebral aneurysmal sac tissue with the aid of polymerase chain reaction (PCR) method. C pneumoniae DNA was searched in 15 surgically clipped and removed aneurysmal sac tissue and in two tumour (an ependymoma of the fourth ventricle and a craniofaringoma) samples by touchdown enzyme time release PCR (TETR PCR) targeting 16S rRNA gene and by nested
PCR targeting ompA gene. Results: Both PCR methods were sensitive to detect in C pneumoniae 4x10^{-2} genomes. C pneumoniae DNA was not detected in any of the 17 sample tissues of these patients. Conclusion: The contribution of C pneumoniae in the development of intracranial aneurysms cannot be excluded despite the results of this study. Further studies on the possible role of C pneumoniae or any other micro-organisms in the pathogenesis of aneurysms should be performed.

J. Neurosci. (32)


http://www.jneurosci.org/cgi/content/abstract/23/6/2333

Estrogens induce synaptogenesis in the CA1 region of the dorsal hippocampus during the estrous cycle of the female rat. Functional consequences of such estrogen-mediated synaptogenesis include cyclic changes in neurotransmission and memory. At the molecular level, estrogen stimulates the rapid activation of specific signal transduction pathways, and of particular interest is the activation of Akt (protein kinase B), a key signal transduction intermediate that initiates protein translation by alleviating the downstream translational repression of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Using a well established in vitro model system of differentiated NG108-15 neurons to investigate such rapid signaling effects of estrogen, we show that estrogen stimulates the phosphorylation of Akt, an indication of kinase activation, as well as the phosphorylation of 4E-BP1. In turn, the activation of these signaling intermediates suggests a non-genomic mechanism by which estrogen might likewise lead to protein translation of dendrite-localized mRNA transcripts in the hippocampus in vivo. We therefore considered the translation of the dendritic spine scaffolding protein postsynaptic density-95 (PSD-95). Although estrogen does not stimulate a rapid increase in PSD-95 mRNA levels in NG108-15 neurons, we show here that estrogen does however stimulate a rapid increase in PSD-95 new protein synthesis in vitro and that this new protein synthesis is Akt dependent. These results demonstrate an essential role for Akt in estrogen-stimulated dendritic spine protein expression, describe for the first time a signal transduction pathway in PSD-95 expression, and delineate a novel, molecular mechanism by which ovarian hormones might translationally regulate synaptogenesis via activating protein synthesis for dendritic function.


http://www.jneurosci.org/cgi/content/abstract/24/40/8796

Although the basic molecular components that promote regulated neurotransmitter release are well established, the contribution of these proteins as regulators of the plasticity of neurotransmission and refinement of synaptic connectivity during development is elaborated less fully. For example, during the period of synaptic growth and maturation in brain, the expression of synaptosomal protein 25 kDa (SNAP-25), a neuronal t-SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) essential for action potential-dependent neuroexocytosis, is altered through alternative splicing of pre-mRNA transcripts. We addressed
the role of the two splice-variant isoforms of SNAP-25 with a targeted mouse mutation that impairs the shift from SNAP-25a to SNAP-25b. Most of these mutant mice die between 3 and 5 weeks of age, which coincides with the time when SNAP-25b expression normally reaches mature levels in brain and synapse formation is essentially completed. The altered expression of these SNAP-25 isoforms influences short-term synaptic function by affecting facilitation but not the initial probability of release. This suggests that mechanisms controlling alternative splicing between SNAP-25 isoforms contribute to a molecular switch important for survival that helps to guide the transition from immature to mature synaptic connections, as well as synapse regrowth and remodeling after neural injury.


http://www.jneurosci.org/cgi/content/abstract/22/13/5403

The chemokine RANTES is critically involved in neuroinflammation and has been implicated in the pathophysiology of multiple sclerosis. We examined the possibility that activation of G-protein-coupled metabotropic glutamate (mGlu) receptors regulates the formation of RANTES in glial cells. A 15 hr exposure of cultured astrocytes to tumor necrosis factor-[alpha] and interferon-[gamma] induced a substantial increase in both RANTES mRNA and extracellular RANTES levels. These increases were markedly reduced when astrocytes were coincubated with L-2-amino-4-phosphonobutanoate (L-AP-4), 4-phosphonophenylglycine, or L-serine-O-phosphate, which selectively activate group III mGlu receptor subtypes (i.e., mGlu4, -6, -7, and -8 receptors). Agonists of mGlu1/5 or mGlu2/3 receptors were virtually inactive. Inhibition of RANTES release produced by L-AP-4 was attenuated by the selective group III mGlu receptor antagonist (R,S)-[alpha]-methylserine-O-phosphate or by pretreatment of the cultures with pertussis toxin. Cultured astrocytes expressed mGlu4 receptors, and the ability of L-AP-4 to inhibit RANTES release was markedly reduced in cultures prepared from mGlu4 knock-out mice. This suggests that activation of mGlu4 receptors negatively modulates the production of RANTES in glial cells. We also examined the effect of L-AP-4 on the development of experimental allergic encephalomyelitis (EAE) in Lewis rats. L-AP-4 was subcutaneously infused for 28 d by an osmotic minipump that released 250 nl/hr of a solution of 250 mM of the drug. Detectable levels of L-AP-4 (~100 nM) were found in the brain dialysate of EAE rats. Infusion of L-AP-4 did not affect the time at onset and the severity of neurological symptoms but significantly increased the rate of recovery from EAE. In addition, lower levels of RANTES mRNA were found in the cerebellum and spinal cord of EAE rats infused with L-AP-4. These results suggest that pharmacological activation of group III mGlu receptors may be useful in the experimental treatment of neuroinflammatory CNS disorders.


http://www.jneurosci.org/cgi/content/abstract/24/23/5410

Wakefulness and sleep are accompanied by changes in behavior and neural activity, as well as by the upregulation of different functional categories of genes. However, the mechanisms responsible for such state-dependent changes in gene expression are unknown. Here we investigate to what extent state-dependent changes in gene expression depend on the central noradrenergic (NA) system, which is active in wakefulness and reduces its firing during sleep. We measured the levels of ~5000 transcripts expressed in the cerebral cortex of control rats and in rats pretreated with DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzyamine], a neurotoxin that removes the noradrenergic innervation of the cortex. We found that NA depletion reduces the
expression of ~20% of known wakefulness-related transcripts. Most of these transcripts are involved in synaptic plasticity and in the cellular response to stress. In contrast, NA depletion increased the expression of the sleep-related gene encoding the translation elongation factor 2. These results indicate that the activity of the central NA system during wakefulness modulates neuronal transcription to favor synaptic potentiation and counteract cellular stress, whereas its inactivity during sleep may play a permissive role to enhance brain protein synthesis.


http://www.jneurosci.org/cgi/content/abstract/23/8/3343

Developing neurons must respond to a wide range of extracellular signals during the process of brain morphogenesis. One mechanism through which immature neurons respond to such signals is by altering cellular actin dynamics. A recently discovered link between extracellular signaling events and the actin cytoskeleton is the WASP/WAVE (Wiscott-Aldrich Syndrome protein/WASP-family verprolin-homologous protein) family of proteins. Through a direct interaction with the Arp2/3 (actin-related protein) complex, this family functions to regulate the actin cytoskeleton by mediating signals from cdc42 as well as other small GTPases. To evaluate the role of WASP/WAVE proteins in the process of neuronal morphogenesis, we used a retroviral gene trap to generate a line of mice bearing a disruption in the WAVE1 gene. Using a heterologous reporter gene, we found that WAVE1 expression becomes increasingly restricted to the CNS over the course of development. Homozygous disruption of the WAVE1 gene results in postnatal lethality. In addition, these animals have severe limb weakness, a resting tremor, and notable neuroanatomical malformations without overt histopathology of peripheral organs. We did not detect any alterations in neuronal morphology in vivo or the ability of embryonic neurons to form processes in vitro. Our data indicate that WAVE1, although important for the general development of the CNS, is not essential for the formation and extension of neuritic processes.


http://www.jneurosci.org/cgi/content/abstract/23/11/4700

Compensatory mechanisms after genetic manipulations have been documented extensively for the nervous system. In many cases, these mechanisms involve genetic regulation at the transcription or expression level of existing isoforms. We report a novel mechanism by which single neurons compensate for changes in network connectivity by retuning their intrinsic electrical properties. We demonstrate this mechanism in the inferior olive, in which widespread electrical coupling is mediated by abundant gap junctions formed by connexin 36 (Cx36). It has been shown in various mammals that this electrical coupling supports the generation of subthreshold oscillations, but recent work revealed that rhythmic activity is sustained in knock-outs of Cx36. Thus, these results raise the question of whether the olivary oscillations in Cx36 knock-outs simply reflect the status of wild-type neurons without gap junctions or the outcome of compensatory mechanisms. Here, we demonstrate that the absence of Cx36 results in thicker dendrites with gap-junction-like structures with an abnormally wide interneuronal gap that prevents electrotonic coupling. The mutant olivary neurons show unusual voltage-dependent oscillations and an increased excitability that is attributable to a combined decrease in leak conductance and an increase in voltage-dependent calcium conductance. Using dynamic-clamp techniques, we demonstrated that these changes are sufficient to transform a wild-type neuron into a knock-out-like neuron. We conclude that the absence of Cx36 in the inferior olive is not
compensated by the formation of other gap-junction channels but instead by changes in the cytological and electroresponsive properties of its neurons, such that the capability to produce rhythmic activity is maintained.


http://www.jneurosci.org/cgi/content/abstract/22/12/4878

Glutamate released onto retinal ON bipolar neurons binds to a metabotropic receptor to activate a heterotrimeric G-protein (Go) that ultimately closes a nonspecific cation channel. Signaling requires the [alpha] subunit (G[alpha]o), but its effector is unknown. Because G[alpha]o is transcribed into two splice variants ([alpha]o1 and [alpha]o2) that differ in the key GTPase domain, the next step in elucidating this pathway was to determine which splice variant carries the signal. Here we show by reverse transcription-PCR and Western blots that retina expresses both splice variants. Furthermore, in situ hybridization and immunostaining on mouse retina deficient in one splice variant or the other show that both [alpha]o1 and [alpha]o2 are expressed by ON bipolar cells but that [alpha]o1 is much more abundant. Finally, electroretinography performed on mice deficient for one splice variant or the other shows that the positive b-wave (response of ON bipolar cells to rod and cone input) requires [alpha]o1 but not [alpha]o2. Thus, the light response of the ON bipolar cell is probably carried by its strongly expressed splice variant, G[alpha]o1.


http://www.jneurosci.org/cgi/content/abstract/24/27/6218

During peripheral nervous system development, Schwann cells are precisely matched to the axons that they support. This is mediated by axonal neuregulins that are essential for Schwann cell survival and differentiation. Here, we show that sensory and motor axons rapidly release heparin-binding forms of neuregulin in response to Schwann cell-derived neurotrophic factors in a dose-dependent manner. Neuregulin release occurs within minutes, is saturable, and occurs from axons that were isolated using a newly designed chamber slide apparatus. Although NGF and glial cell line-derived neurotrophic factor (GDNF) were the most potent neurotrophic factors to release neuregulin from sensory neurons, GDNF and BDNF were most potent for motor neurons and were the predominant neuregulin-releasing neurotrophic factors produced by cultured Schwann cells. Comparable levels of neuregulin could be released at a similar rate from neurons after protein kinase C activation with the phorbol ester, phorbol 12-myristate 13-acetate, which has also been shown to promote the cleavage and release of neuregulin from its transmembrane precursor. The rapid release of neuregulin from axons in response to Schwann cell-derived neurotrophic factors may be part of a spatially restricted system of communication at the axoglial interface important for proper peripheral nerve development, function, and repair.


http://www.jneurosci.org/cgi/content/abstract/24/20/4737
Expression of the brain-gut peptide cholecystokinin (CCK) in the developing olfactory-gonadotropin-releasing hormone-1 (GnRH-1) neuroendocrine systems was characterized, and the function of CCK in these systems was analyzed both in vivo and in vitro. We present novel data demonstrating that CCK transcript and protein are expressed in sensory cells in the developing olfactory epithelium and vomeronasal organ, with both ligand and receptors (CCK-1R and CCK-2R) found on olfactory axons throughout prenatal development. In addition, migrating GnRH-1 neurons in nasal regions express CCK-1R but not CCK-2R receptors. The role of CCK in olfactory-GnRH-1 system development was evaluated using nasal explants, after assessing that the in vivo expression of both CCK and CCK receptors was mimicked in this in vitro model. Exogenous application of CCK (10-7 M) reduced both olfactory axon outgrowth and migration of GnRH-1 cells. This inhibition was mediated by CCK-1R receptors. Moreover, CCK-1R but not CCK-2R antagonism caused a shift in the location of GnRH-1 neurons, increasing the distance that the cells migrated. GnRH-1 neuronal migration in mice carrying a genetic deletion of either CCK-1R or CCK-2R receptor genes was also analyzed. At embryonic day 14.5, the total number of GnRH-1 cells was identical in wild-type and mutant mice; however, the number of GnRH-1 neurons within forebrain was significantly greater in CCK-1R-/- embryos, consistent with an accelerated migratory process. These results indicate that CCK provides an inhibitory influence on GnRH-1 neuronal migration, contributing to the appropriate entrance of these neuroendocrine cells into the brain, and thus represent the first report of a developmental role for CCK.


http://www.jneurosci.org/cgi/content/abstract/22/5/1600

Xenopus laevis retinas, like retinas from all vertebrate classes, have endogenous circadian clocks that control many aspects of normal retinal physiology occurring in cells throughout all layers of the retina. The localization of the clock(s) that controls these various rhythms remains unclear. One of the best studied rhythmic events is the nocturnal release of melatonin. Photoreceptor layers can synthesize rhythmic melatonin when these cells are in isolation. However, within the intact retina, melatonin is controlled in a complex way, indicating that signals from many parts of the retina may contribute to the production of melatonin rhythmicity. To test this hypothesis, we generated transgenic tadpoles that express different levels of a dominant negative Xenopus CLOCK specifically in the retinal photoreceptors. Eyes from these tadpoles continued to produce melatonin at normal levels, but with greatly disrupted rhythmicity, the severity of which correlated with the transgene expression level. These results demonstrate that although many things contribute to melatonin production in vivo, the circadian clock localized in the retinal photoreceptors is necessary for its rhythmicity. Furthermore, these data show that the control of the level of melatonin synthesis is separable from the control of its rhythmicity and may be controlled by different molecular machinery. This type of specific "molecular lesion" allows perturbation of the clock in intact tissues and is valuable for dissection of clock control of tissue-level processes in this and other complex systems.


http://www.jneurosci.org/cgi/content/abstract/22/17/7586

The molecular control mechanisms and regulatory molecules involved in nerve repair are not yet well known. Schwann cells have been attributed an important role in peripheral nerve
regeneration; therefore, attention has been drawn to regulatory factors expressed by these glial cells. Here, we demonstrate that Mash2, a basic helix-loop-helix (bHLH) transcription factor previously shown to be crucial for placenta development, is expressed by Schwann cells of adult peripheral nerves. We observed that this gene is downregulated after nerve lesion and, using cDNA array hybridization technology, we could demonstrate that Mash2 is a regulator of Krox24, Mob-1, and CXCR4 expression in cultured Schwann cells. In addition, we provide strong evidence that Mash2 is a negative regulator of Schwann cell proliferation. Mash2 represents a first candidate for the missing class B bHLH proteins in peripheral nerves.


http://www.jneurosci.org/cgi/content/abstract/24/43/9623

The proinflammatory and lipopolysaccharide (LPS)-inducible cytokine tumor necrosis factor {alpha} (TNF{alpha}) has been shown to enhance primary sensory nociceptive signaling. However, the precise cellular sites of TNF{alpha} and TNF receptor synthesis are still a matter of controversy. Therefore, we differentiated the neuronal and non-neuronal sites of TNF{alpha}, TNFR1, and TNFR2 mRNA synthesis in dorsal root ganglion (DRG) of control rats and evaluated how their expression is altered under systemic challenge with LPS. In situ hybridization (ISH), RT-PCR analysis of laser-microdissected cells, and immunocytochemistry revealed absence of TNF{alpha} from DRG neurons and LPS-induced expression of TNF{alpha} exclusively in a subpopulation of non-neuronal DRG cells. Using RT-PCR and Northern blotting TNFR1 and TNFR2 mRNAs were found to be constitutively expressed and increased after LPS. TNFR1 mRNA was expressed in virtually all neurons and in non-neuronal cells with increased levels after LPS in both. TNFR2 was exclusively expressed and regulated in non-neuronal cells. RT-PCR analysis of microdissected DRG neurons and of the sensory neuronal cell line F11 confirmed the neuronal expression of TNFR1 and excluded that of TNFR2. Double ISH revealed varying levels of TNFR1 mRNA in virtually all DRG neurons including putative nociceptive neurons coding for calcitonin gene-related peptide, substance P, or vanilloid receptor 1. Taken together, we provide evidence that non-neuronally synthesized TNF{alpha} may directly act on primary afferent neurons via TNFR1 but not TNFR2. This is likely to be relevant under conditions of inflammatory pain and infections accompanied by widespread TNF{alpha} synthesis and release and may drive sickness behavior.


http://www.jneurosci.org/cgi/content/abstract/24/12/2866

In an effort to understand the complexity of genomic responses within selectively vulnerable regions after experimental brain injury, we examined whether single apoptotic neurons from both the CA3 and dentate differed from those in an uninjured brain. The mRNA from individual active caspase 3(+)/terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling [TUNEL(-)] and active caspase 3(+)TUNEL(+) pyramidal and granule neurons in brain-injured mice were amplified and compared with those from nonlabeled neurons in uninjured brains. Gene analysis revealed that overall expression of mRNAs increased with activation of caspase 3 and decreased to below uninjured levels with TUNEL reactivity. Cell type specificity of the apoptotic response was observed with both regionally distinct expression of mRNAs and differences in those mRNAs that were maximally regulated. Immunohistochemical analysis for two of the most highly differentially expressed genes (prion and Sos2) demonstrated a correlation between the
observed differential gene expression after traumatic brain injury and corresponding protein translation.


http://www.jneurosci.org/cgi/content/abstract/22/24/10699

Generalized epilepsy with febrile seizures plus type 1 is an inherited human epileptic syndrome, associated with a cysteine-to-tryptophan (C121W) mutation in the extracellular immunoglobin domain of the auxiliary [beta]1 subunit of the voltage-gated sodium channel. The mutation disrupts [beta]1 function, but how this leads to epilepsy is not understood. In this study, we make several observations that may be relevant for understanding why this [beta]1 mutation results in seizures. First, using electrophysiological recordings from mammalian cell lines, coexpressing sodium channel [alpha] subunits and either wild-type [beta]1 or C121W[beta]1, we show that loss of [beta]1 functional modulation, caused by the C121W mutation, leads to increased sodium channel availability at hyperpolarized membrane potentials and reduced sodium channel rundown during high-frequency channel activity, compared with channels coexpressed with wild-type [beta]1. In contrast, neither wild-type [beta]1 nor C121W[beta]1 significantly affected sodium current time course or the voltage dependence of channel activation. We also show, using a Drosophila S2 cell adhesion assay, that the C121W mutation disrupts [beta]1-[beta]1 homophilic cell adhesion, suggesting that the mutation may alter the ability of [beta]1 to mediate protein-protein interactions critical for sodium channel localization. Finally, we demonstrate that neither functional modulation nor cell adhesion mediated by wild-type [beta]1 is occluded by coexpression of C121W[beta]1, arguing against the idea that the mutant [beta]1 acts as a dominant-negative subunit. Together, these data suggest that C121W[beta]1 causes subtle effects on channel function and subcellular distribution that bias neurons toward hyperexcitability and epileptogenesis.


http://www.jneurosci.org/cgi/content/abstract/22/23/10088

Epilepsy is a debilitating disease with a strong genetic component. Positional cloning has identified a few genes for rare monogenic epilepsy syndromes; however, the genetics of common human epilepsies are too complex to be analyzed easily by current techniques. Mouse models of epilepsy can further this analysis by eliminating genetic background heterogeneity and enabling the production of sufficient numbers of offspring. Here, we report that Black Swiss mice have a heretofore unrecognized specific susceptibility to audiogenic seizures. These seizures are characterized by wild running, loss of righting reflex, and tonic flexion and extension, and are followed by a postictal period. The susceptibility to these seizures is developmentally regulated, peaking at 21 d of age and nearly disappearing by adulthood. Interestingly, both the susceptibility to seizures and their developmental regulation appear unrelated to hearing thresholds in the Black Swiss strain and backcrossed progeny. Genetic mapping and linkage analysis of hybrid mice localize the seizure gene, jams1 (juvenile audiogenic monogenic seizures), to a 1.6 (+/-) 0.5 centimorgan (cM) region on mouse chromosome 10, delimited by the gene basigin (Bsg) and marker D10Mit140. Interestingly, the majority of the critical region is syntenic to a region on human chromosome 19p13.3 implicated in a familial form of juvenile febrile convulsions. Cloning the gene for audiogenic seizures in these mice may provide important insight into the fundamental mechanisms for developmentally regulated human epilepsy syndromes.

http://www.jneurosci.org/cgi/content/abstract/22/11/4478

Ciliary neurotrophic factor (CNTF) is a potent protective factor for striatal neurons in animal models of Huntington’s disease (HD). Clinical application of this potential therapeutic still requires the design and optimization of delivery systems. In the case of HD, spatial spread in the vast volume occupied by the striatum and long-term delivery of the factor are particular challenges for these systems. We explored the potential of adenovirus-mediated gene transfer to fulfill these requirements by studying the functional and anatomical effects of single-site striatal delivery of CNTF recombinant vectors in a rat model of HD. In an initial series of experiments, unilateral injections of CNTF adenovirus were performed in rats 10, 30, or 90 d before a 5 d neurotoxic treatment with systemic 3-nitropropionic acid (3NP). Preservation of striatal neurons was observed at all time points, demonstrating temporally extended neuroprotective effects of the CNTF adenovirus. In a second series of experiments, bilateral injections of CNTF adenovirus were performed in the medial aspect of the striatum 10 d before starting 3NP intoxication. Despite placement of the CNTF-producing vector outside the lateral striatal area susceptible to lesion, massive protection of corticostriatopallidal circuits was observed, associated with significant behavioral benefits. This spatial spread of neuroprotection is discussed with reference to the retrograde transport of the adenovirus vector and the anterograde transport of the transgenic CNTF. Overall, adenovirus-mediated CNTF gene transfer appears to be a potentially useful delivery system for widespread, long-term circuit neuroprotection in HD patients.


http://www.jneurosci.org/cgi/content/abstract/22/20/8932

Evidence indicates that gonadotropin releasing hormone-1 [GnRH-1, also known as luteinizing hormone releasing hormone (LHRH)] neurons can exhibit synchronized neuroendocrine secretory activity before entrance into the CNS. In this study, we used calcium imaging to evaluate patterns of activity in individual, embryonic, GnRH-1 neurons as well as population dynamics of GnRH-1 neurons in mouse nasal explants maintained for 1 versus 3 weeks. Independent of age, GnRH-1 neurons displayed significant calcium peaks that synchronized at an interval of ~20 min across multiple GnRH-1 cells within an explant. Acute tetrodotoxin treatment decreased the amplitude of calcium peaks in individual GnRH-1 neurons and the duration but not the frequency of synchronized activity in the population of GnRH-1 neurons. Acute GABAB receptor antagonism increased the frequency of synchronized neuronal activity at both ages, whereas acute GABAA receptor antagonism decreased calcium oscillations in individual GNRH-1 cells as well as synchronization of the calcium pulses within the GnRH-1 population at the 1 week time point to background non-GNRH-1 cell levels. These results indicate that developing GnRH-1 neurons rely heavily on GABAergic signaling to initiate synchronized bouts of activity but thereafter, possess an innate capacity for synchronized activity patterns that are modulated by, but not completely dependent on GABAergic signaling.

To investigate whether activation of mitogen-activated protein kinase (MAPK) in damaged and/or undamaged primary afferents participates in neuropathic pain after partial nerve injury, we examined the phosphorylation of extracellular signal-regulated protein kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK) in the L4 and L5 dorsal root ganglion (DRG) in the L5 spinal nerve ligation (SNL) model. We first confirmed, using activating transcription factor 3 and neuropeptide Y immunoreactivity, that virtually all L4 DRG neurons are spared from axotomy in this model. In the injured L5 DRG, the L5 SNL induced the activation of ERK, p38, and JNK in different populations of DRG neurons. In contrast, in the uninjured L4 DRG, the L5 SNL induced only p38 activation in tyrosine kinase A-expressing small- to medium-diameter neurons. Intrathecal ERK, p38, and JNK inhibitor infusions reversed SNL-induced mechanical allodynia, whereas only p38 inhibitor application attenuated SNL-induced thermal hyperalgesia. Furthermore, the L5 dorsal rhizotomy did not prevent SNL-induced thermal hyperalgesia. We therefore hypothesized that p38 activation in the uninjured L4 DRG might be involved in the development of heat hypersensitivity in the L5 SNL model. In fact, the treatment of the p38 inhibitor and also anti-nerve growth factor reduced SNL-induced upregulation of brain-derived neurotrophic factor and transient receptor potential vanilloid type 1 expression in the L4 DRG. Together, our results demonstrate that the L5 SNL induces differential activation of MAPK in injured and uninjured DRG neurons and, furthermore, that MAPK activation in the primary afferents may participate in generating pain hypersensitivity after partial nerve injury.


Through tropo-myosine-related kinase B (TrkB) receptors, brain-derived neurotrophic factor (BDNF) performs many biological functions such as neural survival, differentiation, and plasticity. T1, an isoform of TrkB receptors that lacks a tyrosine kinase, predominates in the adult mammalian CNS, yet its role remains controversial. In this study, to examine whether T1 transduces a signal and to determine its function, we first performed an affinity purification of T1-binding protein with the T1-specific C-terminal peptide and identified Rho GDP dissociation inhibitor 1 (GDI1), a GDP dissociation inhibitor of Rho small G-proteins, as a signaling protein directly associated with T1. The binding of BDNF to T1 caused Rho GDI1 to dissociate from the C-terminal tail of T1. Astrocytes cultured for 30 d expressed only endogenous T1 among the BDNF receptors. In 30 d cultured astrocytes, Rho GDI1, when dissociated in a BDNF-dependent manner, controlled the activities of the Rho GTPases, which resulted in rapid changes in astrocytic morphology. Furthermore, using 2 d cultured astrocytes that were transfected with T1, a T1 deletion mutant, or cyan fluorescent protein fusion protein of the T1-specific C-terminal sequence, we demonstrated that T1-Rho GDI1 signaling was indispensable for regulating the activities of Rho GTPases and for the subsequent morphological changes among astrocytes. Therefore, these findings indicate that the T1 signaling cascade can alter astrocytic morphology via regulation of Rho GTPase activity.

Cochlear inner hair cells (IHCs) transduce sound-induced vibrations into a receptor potential (RP) that controls afferent synaptic activity and, consequently, frequency and timing of action potentials in the postsynaptic auditory neurons. The RP is thought to be shaped by the two voltage-dependent K+ conductances, IK,f and IK,s, that are carried by large-conductance Ca2+- and voltage-dependent K+ (BK)- and KV-type K+ channels. Using whole-cell voltage-clamp recordings in the acutely isolated mouse cochlea, we show that IHCs display an additional K+ current that is active at the resting membrane potential ([-72 mV) and deactivates on hyperpolarization. It is potently blocked by the KCNQ-channel blockers linopirdine and XE991 but is insensitive to tetraethylammonium and 4-aminopyridine, which inhibit IK,f and IK,s, respectively. Single-cell PCR and immunocytochemistry showed expression of the KCNQ4 subunit in IHCs. In current-clamp experiments, block of the KCNQ current shifted the resting membrane potential by ~7 to [-65 mV and led to a significant activation of BK channels. Using BK channels as an indicator for submembrane intracellular Ca2+ concentration ([Ca2+]i), it is shown that the shift in IHC resting potential observed after block of the KCNQ channels leads to an increase in [Ca2+]i to values [≥1 {micro}M. In conclusion, KCNQ channels set the resting membrane potential of IHCs in the isolated organ of Corti and thus maintain [Ca2+]i at low levels. Destabilization of the resting potential and increase in [Ca2+]i, as may result from impaired KCNQ4 function in IHCs, provide a novel explanation for the progressive hearing loss (DFNA2) observed in patients with defective KCNQ4 genes.


Neurons require Ca2+-dependent gene transcription for their activity-dependent survival, the mechanisms of which have not been fully elucidated yet. Here, we demonstrate that a novel primary response gene, alivin 1 (ali1), is an activity-dependent gene and promotes survival of neurons. Sequence analyses reveal that rat, mouse, and human Ali1 proteins contain seven leucine-rich repeats, one IgC2-like loop and a transmembrane domain, and display homology to Kek and Trk families. Expression of ali1 mRNA in cultured cerebellar granule neurons is rigidly regulated by KCl and/or NMDA concentrations in the culture medium and tightly correlated to depolarization-dependent survival and/or NMDA-dependent survival of the granule neuron. ali1 mRNA expression was regulated at the transcriptional step by the Ca2+ influx through voltage-dependent L-type Ca2+ channels when the cells were stimulated by 25 mM KCl. Expression of ali1 mRNA in cultured cortical neurons was inhibited when their spontaneous electrical activity was blocked by tetrodotoxin. Thus, the expression is neuronal activity dependent. Overexpression of Ali1 in cerebellar granule neurons inhibited apoptosis that was induced by the medium containing 5 mM KCl. The addition of anti-Ali1 antiserum or the soluble putative extracellular Ali1 domain to the 25 mM KCl-supported culture inhibited the survival of the granule neuron. These results suggest that expression of ali1 promotes depolarization-dependent survival of the granule neuron. Mouse ali1 was mapped to a locus [-]55.3 cM from the centromere on chromosome 15 that is syntenic to positional candidate loci for familial Alzheimer’s disease type 5 and Parkinson’s disease 8 on human chromosome 12.

Basic fibroblast growth factor (bFGF) and its major receptor FGF receptor-1 (FGFR-1) play an important role in the development of the cortex. The mechanisms underlying the mitogenic role of bFGF/FGFR-1 signaling have not been elucidated. Intracellular Ca2+ concentrations ([Ca2+]i) in proliferating cortical neuroepithelial cells are markedly dependent on Ca2+ entry (Maric et al., 2000a). The absence of voltage-dependent Ca2+ entry channels, which emerge later, indicates that other membrane mechanisms regulate [Ca2+]i during proliferation. Canonical transient receptor potential (TRPC) family channels are candidates because they are voltage independent and are expressed during CNS development (Strubing et al., 2003). Here, we investigated the involvement of TRPC1 in bFGF-mediated Ca2+ entry and proliferation of embryonic rat neural stem cells (NSCs). Both TRPC1 and FGFR-1 are expressed in the embryonic rat telencephalon and coimmunoprecipitate. Quantitative fluorescence-activated cell sorting analyses of phenotyped telencephalic dissociates show that ~80% of NSCs are TRPC1+, proliferating, and express FGFR-1. Like NSCs profiled ex vivo, NSC-derived progeny proliferating in vitro coexpress TRPC1 and FGFR1. Antisense knock-down of TRPC1 significantly decreases bFGF-mediated proliferation of NSC progeny, reduces the Ca2+ entry component of the Ca2+ response to bFGF without affecting Ca2+ release from intracellular stores or 1-oleoyl-2-acetyl-sn-glycerol-induced Ca2+ entry, and significantly blocks an inward cation current evoked by bFGF in proliferating NSCs. Both Ca2+ influx evoked by bFGF and NSC proliferation are attenuated by Gd3+ and SKF96365 two antagonists of agonist-stimulated Ca2+ entry. Together, these results show that TRPC1 contributes to bFGF/FGFR-1-induced Ca2+ influx, which is involved in self-renewal of embryonic rat NSCs.


Inflammatory mediators not only activate "pain-"sensing neurons, the nociceptors, to trigger acute pain sensations, more important, they increase nociceptor responsiveness to produce inflammatory hyperalgesia. For example, prostaglandins activate Gs-protein-coupled receptors and initiate cAMP- and protein kinase A (PKA)-mediated processes. We demonstrate for the first time at the cellular level that heat-activated ionic currents were potentiated after exposure to the cAMP activator forskolin in rat nociceptive neurons. The potentiation was prevented in the presence of the selective PKA inhibitor PKI14-22, suggesting PKA-mediated phosphorylation of the heat transducer protein. PKA regulatory subunits were found in close vicinity to the plasma membrane in these neurons, and PKA catalytic subunits only translocated to the cell periphery when activated. The translocation and the current potentiation were abolished in the presence of an A-kinase anchoring protein (AKAP) inhibitor. Similar current changes after PKA activation were obtained from human embryonic kidney 293t cells transfected with the wild-type heat transducer protein vanilloid receptor 1 (VR-1). The forskolin-induced current potentiation was greatly reduced in cells transfected with VR-1 mutants carrying point mutations at the predicted PKA phosphorylation sites. The heat transducer VR-1 is therefore suggested as the molecular target of PKA phosphorylation, and potentiation of current responses to heat depends on phosphorylation at predicted PKA consensus sites. Thus, the PKA/AKAP/VR-1 module presents as the molecular correlate of Gs-mediated inflammatory hyperalgesia.

The causes and mechanisms underlying multidrug resistance (MDR) in epilepsy are still elusive and may depend on inadequate drug concentration in crucial brain areas. We studied whether limbic seizures or anticonvulsant drug treatments in rodents enhance the brain expression of the MDR gene (mdr) encoding a permeability glycoprotein (P-gp) involved in MDR to various cancer chemotherapeutic agents. We also investigated whether changes in P-gp levels affect anticonvulsant drug concentrations in the brain. Mdr mRNA measured by RT-PCR increased by 85% on average in the mouse hippocampus 3-24 hr after kainic acid-induced limbic seizures, returning to control levels by 72 hr. Treatment with therapeutic doses of phenytoin or carbamazepine for 7 d did not change mdr mRNA expression in the mouse hippocampus 1-72 hr after the last drug administration. Six hours after seizures, the brain/plasma ratio of phenytoin was reduced by 30% and its extracellular concentration estimated by microdialysis was increased by twofold compared with control mice. Knock-out mice (mdr1a/b [-]/[-]) lacking P-gp protein showed a 46% increase in phenytoin concentrations in the hippocampus 1 and 4 hr after injection compared with wild-type mice. A significant 23% increase was found in the cerebellum at 1 hr and in the cortex at 4 hr. Carbamazepine concentrations were measurable in the hippocampus at 3 hr in mdr1a/b [-]/[-] mice, whereas they were undetectable at the same time interval in wild-type mice. In rats having spontaneous seizures 3 months after electrically induced status epilepticus, mdr1 mRNA levels were enhanced by 1.8-fold and fivefold on average in the hippocampus and entorhinal cortex, respectively. Thus, changes in P-gp mRNA levels occur in limbic areas after both acute and chronic epileptic activity. P-gp alterations significantly affect antiepileptic drugs concentrations in the brain, suggesting that seizure-induced mdr mRNA expression contributes to MDR in epilepsy.

http://www.jneurosci.org/cgi/content/abstract/23/23/8247

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the dysfunction of the nigrostriatal dopaminergic pathway. Although its etiology is not yet fully understood, an interaction of genetic predisposition and environmental factors is frequently discussed. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can evoke PD-like symptoms and neuropathological changes in various species, including mice. It was found repeatedly that mouse strains differ in their susceptibility to MPTP, which might serve as a model for genetic predisposition to neurodegeneration of the nigrostriatal system. In the present study, F2 intercross mice, derived from parental strains with high (C57BL/6J) versus low (BALB/cJ) MPTP susceptibility, were treated with MPTP and phenotyped for dopamine (DA) loss in the neostriatum, a highly sensitive marker of nigrostriatal dysfunction. A subsequent quantitative trait loci analysis revealed a gender-dependent locus for DA loss on chromosome 15 and a putative locus on chromosome 13. A number of potential candidate genes, including the membrane dopamine transporter, are located in the respective areas. Several mechanisms that are possibly involved in the control of the action of MPTP on the nigrostriatal system are discussed.

http://www.jneurosci.org/cgi/content/abstract/24/8/1996

Astrocytes express ionotropic glutamate receptors (GluRs), and recent evidence suggests that these receptors contribute to direct signaling between neurons and glial cells in vivo. Here, we have used functional and molecular analyses to investigate receptor properties in astrocytes of
human hippocampus resected from patients with pharmacoresistant temporal lobe epilepsy (TLE). Histopathological analysis allowed us to distinguish two forms of epilepsy: Ammon's horn sclerosis (AHS) and lesion-associated TLE. Human hippocampal astrocytes selectively expressed the AMPA subtype of ionotropic glutamate receptors. Single-cell RT-PCR found preferential expression of the subunits GluR1 and GluR2 in human astrocytes, and the expression patterns were similar in patients with AHS and lesion-associated epilepsy. The AMPA receptor-specific modulators, cyclothiazide (CTZ) and 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difuoro-phenoxyacetamide (PEPA), were used to investigate splice variant expression. Astrocytes of sclerotic specimens displayed a slower dissociation of CTZ from the receptor and a lower ratio of current potentiation by PEPA to potentiation by CTZ, suggesting enhanced expression of flip receptor variants in AHS versus lesion-associated epilepsy. Real-time PCR and restriction analysis substantiated this presumption by identifying elevated flip-to-flop mRNA ratios of GluR1 in single astrocytes of AHS specimens. These findings imply that in AHS, glutamate may lead to prolonged depolarization of astrocytes, thereby facilitating the generation or spread of seizure activity.


http://www.jneurosci.org/cgi/content/abstract/23/10/4208

Microarray analysis revealed that transcripts for the Axl and Mer receptor tyrosine kinases are expressed at high levels in O4+-immunopanned oligodendrocytes isolated from second trimester human fetal spinal cord. In humans the sole known ligand for the Axl/Rse/Mer kinases is growth arrest-specific gene 6 (Gas6), which in the CNS is secreted by neurons and endothelial cells. We hypothesized that Gas6 is a survival factor for oligodendrocytes and receptor activation signals downstream to the phosphatidylinositol 3 (PI3)-kinase/Akt pathway to increase cell survival in the absence of cell proliferation. To test this hypothesis, we grew enriched human oligodendrocytes for 6 d on a monolayer of NIH3T3 cells stably expressing Gas6. CNP+ oligodendrocytes on Gas6-secreting 3T3 cells had more primary processes and arborizations than those plated solely on 3T3 cells. Also, a twofold increase in CNP+ and MBP+ oligodendrocytes was observed when they were plated on the Gas6-secreting cells. The effect was abolished in the presence of Axl-Fc but remained unchanged in the presence of the irrelevant receptor fusion molecule TrkA-Fc. A significant decrease in CNP+/TUNEL+ oligodendrocytes was observed when recombinant human Gas6 (rhGas6) was administered to oligodendrocytes plated on poly-L-lysine, supporting a role for Gas6 signaling in oligodendrocyte survival during a period of active myelination in human fetal spinal cord development. PI3-kinase inhibitors blocked the anti-apoptotic effect of rhGas6, whereas a MEK/ERK inhibitor had no effect. Thus Gas6 sustains human fetal oligodendrocyte viability by receptor activation and downstream signaling via the PI3-kinase/Akt pathway.


http://www.jneurosci.org/cgi/content/abstract/24/28/6265

Receptor cells of the auditory and vestibular end organs of vertebrates acquire various types of potassium channels during development. Their expression and kinetics can differ along the tonotopic axis as well as in different cell types of the sensory epithelium. These variations can play a crucial role in modulating sensory transduction and cochlear tuning. Whole-cell tight-seal recordings of isolated hair cells revealed the presence of an arachidonic acid-sensitive A-type channel in the short (outer) hair cells of the chicken cochlea. This polyunsaturated fatty acid
blocked the A-current, thereby increasing the amplitude and duration of the voltage response in these cells. We identified the gene encoding this channel as belonging to a member of the Shal subfamily, Kv4.2. Expression of the recombinant channel shows half-activation and inactivation potentials shifted to more positive values relative to native channels, suggesting that the native channel is coexpressed with an accessory subunit. RT-PCR revealed that transcription begins early in development, whereas in situ hybridization showed mRNA expression limited to the intermediate and short hair cells located in specific regions of the adult cochlea. Additional localization, using immunofluorescent staining, revealed clustering in apical-lateral regions of the receptor cell as well as in the cochlear ganglion. These experiments provide evidence that in addition to membrane proteins modulating excitation in these receptor cells, fatty acids contribute to the coding of auditory stimuli via these channels.


http://www.jneurosci.org/cgi/content/abstract/24/1/1

After a brain insult, ATP is released from injured cells and activates microglia. The microglia that are activated in this way then release a range of bioactive substances, one of which is tumor necrosis factor (TNF). The release of TNF appears to be dependent on the P2X7 receptor. The inhibitors 1,4-diamino-2,3-dicyano-1,4-bis[2-amino-phenylthio]butadiene (U0126), anthra[1,9-cd]pyrazol-6(2H)-one (SP600125), and 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)IH-imidazole (SB203580), which target MEK (mitogen-activated protein kinase kinase), JNK (c-Jun N-terminal kinase), and p38, respectively, all potently suppress the production of TNF in ATP-stimulated microglia, whereas the production of TNF mRNA is strongly inhibited by U0126 and SP600125. SB203580 did not affect the increased levels of TNF mRNA but did prevent TNF mRNA from accumulating in the cytoplasm. The ATP-provoked activation of JNK and p38 [but not extracellular signal-regulated kinase (ERK)] could be inhibited by brilliant blue G, a P2X7 receptor blocker, and by genistein and 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-D]pyrimidine, which are general and src-family-specific tyrosine kinase inhibitors, respectively. Most important, we found that treatment of the microglia in neuron-microglia cocultures with the P2X7 agonist 2'-3'-O-(benzoyl-benzoyl) ATP led to significant reductions in glutamate-induced neuronal cell death, and that either TNF-(alpha) converting enzyme inhibitor or anti-TNF readily suppressed the protective effect implied by this result. Together, these findings indicate that both ERK and JNK are involved in the regulation of TNF mRNA expression, that p38 is involved in the nucleocytoplasmic transport of TNF mRNA, and that a PTK (protein tyrosine kinase), possibly a member of the src family, acts downstream of the P2X7 receptor to activate JNK and p38. Finally, our data suggest that P2X7 receptor-activated microglia protect neurons against glutamate toxicity primarily because they are able to release TNF.


http://www.jneurosci.org/cgi/content/abstract/23/11/4491

Acute functional tolerance to ethanol develops during a single exposure to ethanol; it has been suggested to be a predisposing factor for the development of ethanol dependence. Genetic determinants of acute functional tolerance, as well as of ethanol dependence, have been clearly demonstrated. We describe a novel approach that uses a combination of selective breeding (to segregate genes contributing to the phenotype of interest, i.e., acute functional tolerance to the incoordinating effect of ethanol), quantitative trait locus analysis (to define chromosomal regions
associated with acute functional tolerance), and DNA microarray technology (to identify differentially expressed genes in the brains of the selected lines of mice) to identify candidate genes for the complex phenotype of ethanol tolerance. The results indicate the importance of a signal transduction cascade that involves the glutamate receptor (delta)2 protein, the Ephrin B3 ligand, and the NMDA receptor, as well as a transcriptional regulatory protein that may be induced by activation of the NMDA receptor (zinc finger protein 179) and a protein that can modulate downstream responses to NMDA receptor activation (peroxiredoxin), in mediating acute tolerance to the incoordinating effect of ethanol.


http://www.jneurosci.org/cgi/content/abstract/24/1/138

Mutations in the genes encoding the CNGA3 and CNGB3 subunits of the cyclic nucleotide-gated (CNG) channel of cone photoreceptors have been associated with autosomal recessive achromatopsia. Here we analyze the molecular basis of achromatopsia in two siblings with residual cone function. Psychophysical and electroretinographic analyses show that the light sensitivity of the cone system is lowered, and the signal transfer from cones to secondary neurons is perturbed. Both siblings carry two mutant CNGA3 alleles that give rise to channel subunits with different single-amino acid substitutions. Heterologous expression revealed that only one mutant forms functional channels, albeit with grossly altered properties, including changes in Ca2+ blockage and permeation. Surprisingly, coexpression of this mutant subunit with CNGB3 rescues the channel phenotype, except for the Ca2+ interaction. We argue that these alterations are responsible for the perturbations in light sensitivity and synaptic transmission.


http://www.jneurosci.org/cgi/content/abstract/25/4/778

Recent studies have begun to focus on the signals that regulate axonal protein synthesis and the functional significance of localized protein synthesis. However, identification of proteins that are synthesized in mammalian axons has been mainly based on predictions. Here, we used axons purified from cultures of injury-conditioned adult dorsal root ganglion (DRG) neurons and proteomics methodology to identify axonally synthesized proteins. Reverse transcription (RT)-PCR from axonal preparations was used to confirm that the mRNA for each identified protein extended into the DRG axons. Proteins and the encoding mRNAs for the cytoskeletal proteins {beta}-actin, peripherin, vimentin, {gamma}-tropomyosin 3, and coflin 1 were present in the axonal preparations. In addition to the cytoskeletal elements, several heat shock proteins (HSP27, HSP60, HSP70, grp75, {alpha}B crystallin), resident endoplasmic reticulum (ER) proteins (calreticulin, grp78/BiP, ERp29), proteins associated with neurodegenerative diseases (ubiquitin C-terminal hydrolase L1, rat ortholog of human DJ-1/Park7, {gamma}-synuclein, superoxide dismutase 1), anti-oxidant proteins (peroxiredoxins 1 and 6), and metabolic proteins (e.g., phosphoglycerate kinase 1 (PGK 1), {alpha} enolase, aldolase C/Zebrin II) were included among the axonally synthesized proteins. Detection of the mRNAs encoding each of the axonally synthesized proteins identified by mass spectrometry in the axonal compartment indicates that the DRG axons have the potential to synthesize a complex population of proteins. Local treatment of the DRG axons with NGF or BDNF increased levels of cytoskeletal mRNAs into the axonal compartment by twofold to fivefold but had no effect on levels of the other axonal mRNAs studied. Neurotrophins selectively increased transport of {beta}-actin, peripherin, and vimentin mRNAs from the cell body into the axons rather than changing transcription or mRNA survival in
Studies increasingly indicate that dietary indole-3-carbinol (I3C) prevents the development of estrogen-enhanced cancers including breast, endometrial and cervical cancers. Epidemiological, laboratory, animal and translational studies support the efficacy of I3C. Whereas estrogen increases the growth and survival of tumors, I3C causes growth arrest and increased apoptosis and ameliorates the effects of estrogen. Our long-range goal is to best use I3C together with other nutrients to achieve maximum benefits for cancer prevention. This study examines the possibility that induction of growth arrest in response to DNA damage (GADD) in genes by diindolylmethane (DIM), which is the acid-catalyzed condensation product of I3C, promotes metabolically stressed cancer cells to undergo apoptosis. We evaluated whether genistein, which is the major isoflavonoid in soy, would alter the ability of I3C/DIM to cause apoptosis and decrease expression driven by the estrogen receptor (ER)-α. Expression of GADD was evaluated by real-time reverse transcription-polymerase chain reaction. Proliferation and apoptosis were measured by a mitochondrial function assay and by fluorescence-activated cell sorting analysis. The luciferase reporter assay was used to specifically evaluate expression driven by ER-α. The estrogen-sensitive MCF-7 breast cancer cell line was used for these studies. We show a synergistic effect of I3C and genistein for induction of GADD expression, thus increasing apoptosis, and for decrease of expression driven by ER-α. Because of the synergistic effect of I3C and genistein, the potential exists for prophylactic or therapeutic efficacy of lower concentrations of each phytochemical when used in combination.

http://www.nutrition.org/cgi/content/abstract/132/8/2393S

This study was designed to determine if maternal dietary methyl supplements increase DNA methylation and methylation-dependent epigenetic phenotypes in mammalian offspring. Female mice of two strains were fed two levels of dietary methyl supplement or control diet prior to and during pregnancy. Offspring of these mice vary in phenotype, which is epigenetically determined and affects health and 2-y survival. Phenotype and DNA methylation of a long terminal repeat (LTR) controlling expression of the agouti gene were assayed in the resulting offspring. Methyl supplements increase the level of DNA methylation in the agouti LTR and change the phenotype of offspring in the healthy, longer-lived direction. This shows that methyl supplements have strong effects on DNA methylation and phenotype and are likely to affect long-term health. Optimum dietary supplements for the health and longevity of offspring should be intensively investigated. This should lead to public policy guidance that teaches optimal, rather than minimal, dose levels of maternal supplements.

http://www.nutrition.org/cgi/content/abstract/132/8/2457S

Periconceptional folic acid supplementation has been shown to prevent up to 70% of neural tube and other birth defects in humans; however, the mechanism is still unknown. In this study, we tested whether defective intracellular folate transport, as achieved by inactivation of the murine folate-binding protein 1 (Folbp1), affects global DNA methylation in the liver and brain from gestational day (GD) 15 embryos. Complete Folbp1 inactivation is embryolethal but can be reversed by maternal folinic acid (FA) supplementation, and thus we also tested the effect of FA supplementation on DNA methylation in Folbp1 fetuses. Overall, the extent of global DNA methylation seems to be similar across all genotypes in unsupplemented control Folbp1 mice; however, explicit conclusions regarding Folbp1-/- fetuses were not possible because only a single living unsupplemented fetus was viable at GD 15. FA supplementation induced global DNA hypomethylation across all genotypes. FA-induced hypomethylation is most likely due to its ability to inhibit the enzyme glycine hydroxymethyltransferase, thereby inhibiting the homocysteine remethylation cycle necessary to regenerate S-adenosylmethionine, the methyl donor for DNA methyltransferases. Our hypothesis was that due to defective folate transport in Folbp1-/- embryos and fetuses, DNA would be hypomethylated, thereby altering the temporal expression of critical genes necessary for normal embryonic development. However, these results suggest that an extended examination of changes in DNA methylation prior to GD 15 is required to unequivocally prove or disprove the hypothesis.


http://www.nutrition.org/cgi/content/abstract/135/1/33

We reported previously that genistein enhances the expression of genes involved in fatty acid catabolism through activation of peroxisome proliferator-activated receptor (PPAR) {alpha} in HepG2 cells, suggesting that genistein holds great promise for therapeutic applications to lipid abnormalities such as obesity and hyperlipidemia in humans. In this study, we examined the changes in hepatic transcriptional profiles using cDNA microarrays in mice with high-fat diet (HFD)-induced obesity supplemented with genistein. C57BL/6J male mice (n = 10/group) were fed a low-fat diet (LFD), a HFD, or a HFD supplemented with 2 g/kg genistein (HFD+GEN) for 12 wk. Mice fed the HFD had abnormal lipid profiles and significantly greater body weight and visceral fat accumulation than the LFD-fed group. Genistein supplementation improved lipid profiles and hepatic steatosis and attenuated the increases in body weight and visceral fat in HFD-fed mice. The cDNA microarrays revealed marked alterations in the expression of 107 genes in the mice fed the HFD and/or the HFD+GEN. Of 97 transcripts altered in the HFD-fed group, 84 genes were normalized by genistein supplementation. However, several genes involved in fatty acid catabolism were not normalized but were still upregulated in the HFD+GEN-fed group, relative to the LFD-fed group. Furthermore, carnitine O-octanoyltransferase, which accelerates fatty acid oxidation, was not affected by the HFD, but was induced by genistein supplementation. These results are consistent with our previous study showing that genistein is an activator of PPAR {alpha} in vitro. This study showed beneficial effects of genistein supplementation in preventing the development of obesity and metabolic abnormalities in mice with diet-induced obesity. Our results also provide interesting information about the genes associated with the beneficial effects of genistein as well as the mechanisms underlying the development and maintenance of the obesity phenotype in vivo.

http://www.nutrition.org/cgi/content/abstract/132/6/1129

The effects of water-soluble organosulfur compounds of garlic on hepatic cholesterol biosynthesis in cultured rat hepatocytes were studied. S-Alk(en)yl cysteines, i.e., S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) inhibited cholesterol synthesis from [14C]acetate but not from [14C]mevalonate. The activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the cells treated with SAC, SEC and SPC was 30-40% lower than that of the untreated cells. S-Alk(en)yl cysteines did not alter abundance of mRNA coded for HMG-CoA reductase or protein concentration of the enzyme. The ratio of expressed to total activity (E/T) of HMG-CoA reductase was then determined as an index of phosphorylation status of the enzyme. The E/T ratio was reduced 18-29% by SAC, SEC and SPC, resulting primarily from decreased expressed activity. The results suggest that S-alk(en)yl cysteines inhibit cholesterol synthesis by deactivating HMG-CoA reductase via enhanced phosphorylation, but not changing levels of mRNA or the amount of the enzyme. Additionally, of the three S-alk(en)yl cysteines tested, only SAC appears to further decrease the activity of HMG-CoA reductase by increasing sulfhydryl oxidation of the enzyme.


http://www.nutrition.org/cgi/content/abstract/132/10/3036

Lignans are plant compounds metabolized in the gut to produce the phytoestrogens enterolactone and enterodiol. Reduced breast cancer risks associated with higher urinary lignan excretion may be related to competitive inhibition of endogenous estrogens. Evidence exists that associations with reproductive risk factors for breast cancer differ according to cytochrome P450c17(alph) (CYP17) genotype. Genetic variability in estrogen metabolism could affect lignan metabolism thereby modifying risk associations. We examined breast cancer risk, dietary lignans and CYP17 genotype among 207 women with primary, incident, histologically confirmed breast cancer and 188 controls frequency matched to cases by age and county of residence. Self-reported frequency of intake of 170 foods and beverages during the 2 y before the interview and other relevant data were collected by detailed in-person interviews. Dietary lignan intake was expressed as the sum of enterolactone and enterodiol production from foods. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression, adjusting for age, education and other breast cancer risk factors. Women in the highest tertile of dietary lignans tended to have reduced breast cancer risk (OR 0.45, 95% CI 0.20-1.01 and OR 0.59, 95% CI 0.28-1.27, pre- and postmenopausal women, respectively). Substantially reduced risks in the highest tertile of lignans were observed for premenopausal women with at least one A2 allele (OR 0.12, 95% CI 0.03-0.50). Our results suggest that CYP17 genotype may be important in modifying the effect on breast cancer risk of exogenous estrogens, particularly for premenopausal women.


http://www.nutrition.org/cgi/content/abstract/132/4/652
Dietary nucleotides reportedly promote functionality and repair in fibrotic liver. Liver fibrosis is characterized by an excessive accumulation of extracellular matrix components, which lead to the impairment of the hepatic function. The aim of this work was to evaluate the influence of dietary nucleotides on liver fibrosis induced by thioacetamide and to elucidate the mechanism by which nucleotides exert their protective effects. Rats consumed ad libitum 300 mg/L thioacetamide in drinking water and were pair-fed diets with (group TN) or without nucleotides (group TS) for 4 mo. Liver histology and extracellular matrix components, liver collagenase and prolyl 4-hydroxylase activities, and tissue inhibitor of metalloproteinases-1 were assessed. The degree of fibrosis was lower in group TN than in group TS. Group TN had lower hepatic concentration of hydroxyproline (P < 0.05), collagen type I (P = 0.12) and type III (P = 0.20), fibronectin (P = 0.05), laminin (P = 0.11) and desmin (P = 0.07), higher collagenolytic activity (P < 0.05), lower prolyl 4-hydroxylase activity (P < 0.05) and lower prolyl 4-hydroxylase and tissue inhibitor of metalloproteinase-1 (P = 0.10) expression than group TS. Moreover, expression of tissue inhibitor of the metalloproteinases-1 gene was lower in group TN than in group TS (P < 0.05). These data indicate that the reduction of liver fibrosis in nucleotide-supplemented rats may rely on the enhancement of collagenase activity and the reduction of collagen content and maturation.


http://www.nutrition.org/cgi/content/abstract/135/4/837

We showed previously that fructooligosaccharides (FOS) decrease the resistance to salmonella infection in rats. However, the mechanism responsible for this effect is unclear. Therefore, we examined whether dietary FOS affects intestinal permeability before and after infection with Salmonella enterica serovar Enteritidis. Male Wistar rats were fed restricted quantities of a purified diet that mimicked the composition of a Western human diet. The diet was supplemented with 60 g/kg cellulose (control) or 60 g/kg FOS and with 4 mmol/kg of the intestinal permeability marker chromium EDTA (CrEDTA) (n = 8 or 10). After an adaptation period of 2 wk, rats were orally infected with 108 colony-forming units (cfu) of S. enteritidis. Mucin concentrations in intestinal contents and mucosa were measured fluorimetrically, as markers of mucosal irritation. Intestinal permeability was determined by measuring urinary CrEDTA excretion. Translocation of salmonella was quantified by analysis of urinary nitric oxide metabolites with time. Before infection, FOS increased mucosal lactobacilli and enterobacteria in cecum and colon, but not in the ileum. However, FOS increased cytotoxicity of fecal water and intestinal permeability. Moreover, FOS increased fecal mucin excretion and mucin concentrations in cecal and colonic contents, and in cecal mucosa before infection. After infection, mucin excretion and intestinal permeability in the FOS groups increased even further in contrast to the control group. In addition, FOS increased translocation of salmonella to extraintestinal sites. Thus, FOS impairs the intestinal barrier in rats, as indicated by higher intestinal permeability. Whether these results can be extrapolated to humans requires further investigation.


http://www.nutrition.org/cgi/content/abstract/132/9/2644

Hormonal regulation of calcium (Ca) absorption was investigated in a cholecalciferol (vitamin D3)-supplemented group (hVitD) vs. a control group (cVitD) of growing Great Danes (100 vs. 12.5 {micro}g vitamin D3/kg diet). Although Ca intakes did not differ, fractional Ca absorption was significantly lower in the hVitD group than in the cVitD group. There were no differences in plasma concentrations of Ca, inorganic phosphate, parathyroid hormone, growth hormone or
insulin-like growth factor I between groups. Plasma 25-hydroxycholecalciferol \([25(\text{OH})\text{D}3]\) concentrations were maintained in the hVitD dogs at the same levels as in the cVitD dogs due to increased turnover of 25(\text{OH})\text{D}3 into 24,25-dihydroxycholecalciferol \([24,25(\text{OH})\text{D}3]\) and 1,25-dihydroxycholecalciferol \([1,25(\text{OH})\text{D}3]\). In hVitD dogs, the greater plasma 24,25(\text{OH})\text{D}3 concentration and the enhanced metabolic clearance rate (MCR) of 1,25(\text{OH})\text{D}3 indicated upregulated 24-hydroxylase activity. The increased MCR of 1,25(\text{OH})\text{D}3 decreased plasma 1,25(\text{OH})\text{D}3 concentrations. In hVitD dogs, the greater production rate of 1,25(\text{OH})\text{D}3 was consistent with the 12.9-fold greater renal \{\text{alpha}\}-hydroxylase gene expression compared with cVitD dogs and compensated to a certain extent for the accelerated MCR of 1,25(\text{OH})\text{D}3. The moderately decreased plasma 1,25(\text{OH})\text{D}3 concentration can only partially explain the decreased Ca absorption in the hVitD dogs. Intestinal vitamin D receptor concentrations did not differ between groups and did not account for the decreased Ca absorption. We suggest that 24,25(\text{OH})\text{D}3 may downregulate Ca absorption.

\[\text{J. Pharmacol. Exp. Ther.} (17)\]


http://jpet.aspetjournals.org/cgi/content/abstract/304/3/1299

We hypothesized that the up-regulated expression of one or more members of the regulator of G protein signaling (RGS) family can cause an attenuation of signaling via \text{Gi}/\text{Go}-coupled opioid receptors, and thereby play a role in the development of hyperalgesia and accompanying insensitivity to morphine observed in animal models of neuropathic pain. Accordingly, we examined the mRNA expression of several RGS genes in a rat model of chronic neuropathic pain induced by partial ligation of the sciatic nerve. During the development of hyperalgesia, RGS4 was the only isoform examined whose mRNA levels increased significantly (up to 230\%) in the lumbar spinal cord. In situ hybridization studies confirmed that RGS4 is present in the dorsal horn of the spinal cord where \{\text{micro}\}-opioid receptors (MORs) are also expressed. Overexpression of RGS4 in human embryonic kidney 293 cells stably expressing \{\text{micro}\}-opioid receptors predictably attenuated opioid agonist-induced inhibition of adenylyl cyclase. This inhibitory effect was overcome partially at high agonist concentrations, supporting the view that morphine insensitivity is promoted by RGS4 overexpression. These studies provide evidence that the up-regulation of RGS4 expression may contribute to changes in pain signal processing that lead to the development of hyperalgesia, and further affect its modulation by morphine.


http://jpet.aspetjournals.org/cgi/content/abstract/jpet.105.084855v1

Muscarinic acetylcholine receptors (mAChRs) expressed by pancreatic acinar cells play an important role in mediating acetylcholine-dependent stimulation of digestive enzyme secretion. To examine the potential roles of M1 and M3 mAChRs in this activity, we used M1 and M3 receptor
single knockout (KO) and M1/M3 receptor double KO mice as novel experimental tools. Specifically, we examined the ability of the muscarinic agonist, carbachol, to stimulate amylase secretion in vitro, using dispersed pancreatic acini prepared from wild-type and mAChR mutant mice. Quantitative RT-PCR studies using RNA prepared from mouse pancreatic acini showed that deletion of the M1 or M3 mAChR genes did not lead to significantly altered mRNA levels of the remaining mAChR subtypes. Moreover, immunoprecipitation studies with M1 and M3 mAChR-selective antisera demonstrated that both mAChR subtypes are expressed by mouse pancreatic acini. Strikingly, carbachol-induced stimulation of amylase secretion was significantly impaired in acinar preparations from both M1 and M3 receptor single KO mice and completely abolished in acinar preparations from M1/M3 receptor double KO mice. However, another pancreatic secretagogue, bombesin, retained its ability to fully stimulate amylase secretion in acinar preparations from M1/M3 receptor double KO mice. Taken together these studies support the concept that cholinergic stimulation of pancreatic amylase secretion is mediated by a mixture of M1 and M3 mAChRs and that other mAChR subtypes do not make a significant contribution to this activity. These findings clarify the long-standing question regarding the molecular nature of the mAChR subtypes mediating the secretion of digestive enzymes from the exocrine pancreas.


http://jpet.aspetjourna ls.org/cgi/content/abstract/jpet.105.084699v1

The role of heme in the phenobarbital-mediated induction of CYP2B1/2 was reexamined in rat hepatocytes in monolayer culture, acutely depleted of heme by treatment with either DDEP (3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine) or NMPP (N-methylprotoporphyrins). The findings revealed that such acute hepatic heme depletion markedly impaired CYP2B1/2 protein induction, an effect that was reversible by heme resupplementation. However, TaqMan analyses of hepatic mRNA isolated from these heme depleted cells revealed that this impairment was not due to faulty transcriptional activation of either CYP2B1 or CYP2B2 gene expression as previously proposed, thereby confirming literature reports that heme is not a transcriptional regulator of the CYP2B1/2 gene. In contrast, the rate of de novo CYP2B1/2 protein synthesis was found to be dramatically inhibited in both DDEP- and NMPP-treated hepatocytes. Concurrently, a marked (>80%) suppression of de novo hepatocellular protein synthesis was also observed, along with a significantly enhanced phosphorylation of the {alpha}-subunit of the eukaryotic initiation factor eIF2 (eIF2{alpha}), as monitored by the phosphorylated eIF2{alpha}/total eIF2{alpha} ratio in these heme-depleted cells. Indeed, the parallel reversal of all these three effects by heme supplementation suggests that this impaired CYP2B1 induction most likely stems from blocked translational initiation resulting from the activation of a heme-sensitive eIF2{alpha} kinase. Such global suppression of hepatic protein synthesis may disrupt a myriad of vital cellular functions thereby contributing to the clinical symptoms of acute hepatic heme deficient states such as the hepatic porphyrias.


http://jpet.aspetjournals.org/cgi/content/abstract/312/3/884

Epigallocatechin-3-gallate (EGCG), the most abundant polyphenol in green tea, exerts chemopreventive effects by selectively inducing apoptosis in tumor cells. In contrast, EGCG accelerates terminal differentiation in normal human epidermal keratinocytes (NHEK) mediated
partially by up-regulation of p57/KIP2, a cyclin-dependent kinase inhibitor that confers growth arrest and differentiation. However, it is unclear if EGCG modulates caspase 14, a unique regulator of epithelial cell terminal differentiation associated with cornification. Here, we examined the effect of EGCG on caspase 14 expression in NHEK and correlated the protein and mRNA expression of p57/KIP2 with those of caspase 14 in either normal keratinocytes or p57/KIP2-expressing tumor cells (OSC2, an oral squamous cell carcinoma cell line). Additionally, paraffin-embedded normal and untreated psoriatic (aberrant keratinization) skin sections from humans were assessed for caspase 14 by immunohistochemistry. In NHEK, EGCG induced the expression of caspase 14 mRNA and protein levels within a 24-h period. The expression of p57/KIP2 in OSC2 cells was adequate to induce caspase 14 in the absence of EGCG; this induction of caspase 14 was down-regulated by transforming growth factor-β1. In human psoriatic skin samples, caspase 14 staining in the upper epidermis was reduced, especially in nuclear areas. These results suggest that, in addition to p57/KIP2, EGCG-induced terminal differentiation of epidermal keratinocytes involves up-regulation of caspase 14. Further understanding of how EGCG modulates cellular differentiation may be useful in developing green tea preparations for selected clinical applications.


http://jpet.aspetjournals.org/cgi/content/abstract/309/1/8

To test the hypothesis that estrogen confers cardioprotection by suppressing the expression of β1-adrenoceptor (β1-AR), we first correlated the infarct size in response to ischemic insult and β1-AR stimulation with the expression of β1-AR in sham, ovariectomized (Ovx) and estrogen replaced (Ovx + E2) rats. When β1-AR is being activated during ischemia, the infarct size was significantly greater in Ovx than in the sham and Ovx + E2 rats. There is a negative correlation between the infarct size and the expression level of β1-AR as revealed by Western blotting and supported by binding analysis. Incubation of ventricular myocytes from Ovx rats with estrogen at 10-9 M for 24 and 48 h, but not 12 h, significantly reduced lactate dehydrogenase release when the myocytes are subjected to simulated ischemia. The cardioprotective effect of 24 h estrogen incubation was accompanied by a reduction in the protein expression level of β1-AR, which is estrogen receptor-dependent, whereas the lack of protection of 12-h estrogen incubation was not accompanied by any alterations in the expression level of β1-AR. Together, the result from present study suggested that it is most likely that the cardioprotective effect of long-term estrogen replacement is due to suppressing the enhanced expression of cardiac β1-AR in the Ovx rats, which in turn reduces cardiac injury when β1-AR is activated by sympathetic hyperactivity during ischemia. Therefore, suppression of the enhanced expression of cardiac β1-AR in Ovx rats represents a novel cardioprotective mechanism of estrogen replacement therapy.


http://jpet.aspetjournals.org/cgi/content/abstract/313/1/302

Genetic variation in CYP3A activity may influence the rate of the metabolism and elimination of CYP3A substrates in humans. We previously reported four new CYP3A4 coding variants in three different racial groups. In the present study, we examined metabolism of nifedipine by the recombinant forms of these allelic variants. Metabolism of nifedipine by the L293P (CYP3A4*18),
M445T (CYP3A4*3), and P467S (CYP3A4*19) allelic variants was not significantly different from wild-type CYP3A4*1. However, F189S (CYP3A4*17) exhibited a >99% decrease in both Vmax and CLmax of nifedipine compared with CYP3A4*1. Of 72 racially diverse individuals, CYP3A4*17 was identified in 1 of 24 Caucasian samples [1:5 Eastern European (Adygei ethnic group)]. Genotyping of an extended set of 276 genomic DNAs of Caucasians (100 from the Coriell Repository and an additional 176 from the United States) for CYP3A4*17 detected no additional individuals containing the CYP3A4*17 allele. However, additional genotyping of four more Adygei samples available from Coriell detected an additional individual carrying the CYP3A4*17 allele. New specific polymerase chain reaction-restriction fragment length polymorphism genotyping procedures were developed for the major splice variant of CYP3A5 (CYP3A5*3) and CYP3A4*17. Genotyping revealed that the two individuals carrying CYP3A4*17 were either homozygous or heterozygous for the more frequent CYP3A5*3 allele, suggesting that the two alleles may exist on the same chromosome as a new putative CYP3A poor metabolizer haplotype. We predict that individuals who are homozygous for defective alleles of both of these genes would metabolize CYP3A substrates poorly. The new genetic tests will be useful in future clinical studies to investigate genotype/phenotype associations.


http://jpet.aspetjournals.org/cgi/content/abstract/305/2/515

We investigated the effects of ursodeoxycholic acid (UDCA; 60 {micro}g/day/100 g b.wt.) on the impairment induced by maternal obstructive cholestasis during pregnancy (OCP) in the rat placenta-maternal liver tandem excretory pathway. A blunted catheter was implanted in the common bile duct on day 14 of pregnancy, and the tip was cut on day 21. [14C]Glycocholate (GC) was then administered through the umbilical artery of "in situ" perfused placenta (placental transfer test) or through the maternal jugular vein (biliary secretion test), and GC bile output was measured. OCP impaired both GC placental transfer and maternal biliary secretion. UDCA moderately improved the latter but had a more marked beneficial effect on GC placental transfer. Histological examination revealed trophoblast atrophy and structural alterations, e.g., loss of apical membrane microvilli in OCP placentas. Gene expression level was investigated by real-time quantitative reverse transcription-polymerase chain reaction and Western blot analysis. OCP reduced both placental lactogen II (a trophoblast-specific gene) mRNA and the functional amount of epithelial tissue, determined by transplacental diffusion of antipyrin. Using a rapid filtration technique, impairment in the ATP-dependent GC transport across trophoblast apical plasma membranes obtained from OCP placentas was found. UDCA partially prevented all these changes. The expression level of organic anion transporters Oatp1, Oatp2, and Oatp4, and multidrug resistance-associated proteins Mrp1, Mrp2, and Mrp3 in whole placenta were not affected or were moderately affected by OCP but greatly enhanced by UDCA. In summary, UDCA partially prevents deleterious effects of OCP on the rat placenta-maternal liver tandem excretory pathway, mainly by preserving trophoblast structure and function.


http://jpet.aspetjournals.org/cgi/content/abstract/306/2/638

Vasoactive intestinal peptide receptors 1 (VPAC1) and 2 (VPAC2) have been identified in humans. Cell lines expressing only VPAC1 (HT-29) or VPAC2 (Molt-4b) were identified using
real-time reverse transcriptase polymerase chain reaction. Vasoactive intestinal peptide (VIP) and related peptides, VIP-6-28, VIP4-28, and VIP10-28, previously isolated from cultures of human leukocytes, were evaluated for their ability to bind to VPAC1 and VPAC2 and to increase the levels of cAMP in HT-29 and Molt-4b cells. VIP bound to membranes of HT-29 colon carcinoma cells and Molt-4b lymphoblasts with high affinity (KD = 1.6 {±} 0.2 and 1.7 {±} 0.9 nM, respectively). VIP4-28 also demonstrated high-affinity binding (KD = 1.7 {±} 0.2 and 1.7 {±} 0.7 nM in HT-29 and Molt-4b, respectively). VIP and VIP4-28 are potent VPAC1 agonists, inducing maximal 200- and 400-fold increases in cAMP, respectively. VIP demonstrated weak VPAC2 agonist activity, inducing a maximal 14-fold increase in cAMP. VIP4-28 had no VPAC2 agonist activity but demonstrated potent VPAC2 antagonist activity. VIP4-28 inhibited VPAC2-mediated increases in cAMP in Molt-4b cells up to 95%, but had no antagonistic effect on VPAC1. Lymphoblasts did not hydrolyze VIP4-28 to a form with VPAC1 antagonist activity. VIP4-28 thus is a lymphocyte-generated VIP fragment with potent agonist activity for VPAC1 and potent antagonist activity for VPAC2.


http://jpet.aspetjournals.org/cgi/content/abstract/311/3/1179

Recently, a number of nucleotide variants have been described in the multidrug resistance 1 (MDR1/ABCB1) gene; however, most studies have focused on the coding region. In the present study, we identified promoter variants of the MDR1 gene and evaluated their phenotypic consequences using a reporter gene assay and the real-time polymerase chain reaction method. Ten allelic variants were detected in the promoter region (approximately 2 kilobases), seven of which were newly identified. Certain mutations occurred simultaneously, and a total of 10 haplotypes were observed. These promoter polymorphisms were found more frequently in Japanese than Caucasians. Some haplotypes were associated with changes in luciferase activity and placental and hepatic mRNA levels. We also determined DNA methylation status in the proximal promoter region of the MDR1 gene. The promoter region around potential binding sites for transcription factors was found to be hypomethylated and thus likely to be independent of the gene expression. Nucleotide and/or haplotype variants not only in the coding region but also in the promoter region of the MDR1 gene may be important for interindividual differences of P-glycoprotein expression.


http://jpet.aspetjournals.org/cgi/content/abstract/313/1/250

BL-1249 [(5,6,7,8-tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine] produced a concentration-dependent membrane hyperpolarization of cultured human bladder myocytes, assessed as either a reduction in fluorescence of the voltage-sensitive dye bis-(1,2-dibutylbarbituric acid)trimethine oxonol (EC50 = 1.26 {±} 0.6 {micro}M) or by direct electrophysiological measurement (EC50 = 1.49 {±} 0.08 {micro}M). BL-1249 also produced a membrane hyperpolarization of acutely dissociated rat bladder myocytes. Voltage-clamp studies in human bladder cells revealed that BL-1249 activated an instantaneous, noninactivating current that reversed near EK. The BL-1249-evoked outward K+ current was insensitive to blockade by glyburide, tetraethylammonium, iberiotoxin, 4-aminopyridine, apamin, or Mg2+. However, the current was inhibited by extracellular Ba2+ (10 mM). In in vitro organ bath experiments, BL-1249
produced a concentration-dependent relaxation of 30 mM KCl-induced contractions in rat bladder strips (EC50 = 1.12 +/- 0.37 [micro]M), yet had no effect on aortic strips up to the highest concentration tested (10 [micro]M). The bladder relaxation produced by BL-1249 was partially blocked by Ba2+ (1 and 10 mM) but not by apamin, iberiotoxin, 4-aminopyridine, glyburide, or tetraethylammonium. In an anesthetized rat model, BL-1249 (1 mg/kg i.v.) decreased the number of isovolumic contractions, without significantly affecting blood pressure. Thus, BL-1249 behaves as a potassium channel activator that exhibits bladder versus vascular selectivity both in vitro and in vivo. A survey of potassium channels exhibiting sensitivity to extracellular Ba2+ at millimolar concentration revealed that the expression of the K2P2.1 (TREK-1) channel was relatively high in human bladder cells versus human aortic cells, suggesting this channel as a possible candidate target for BL-1249.


http://jpet.aspetjournals.org/cgi/content/abstract/310/2/783

Mu opioid receptors are present throughout the central and peripheral nervous systems. Peripheral inflammation causes an increase in mu receptor levels on peripheral terminals of primary afferent neurons. Recent studies indicate that activation of peripheral mu receptors produces antihyperalgesic effects in animals and humans. Here, we describe the in vitro pharmacological and in vivo pharmacokinetic properties of a novel, highly potent, and peripherally restricted mu opioid agonist, [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl]-acetic acid (DiPOA). In a radioligand binding assay, DiPOA inhibited [3H]-diprenorphine binding to recombinant human mu receptors with a Ki value of [-7]0.8 nM. The rank order of affinity for DiPOA binding to recombinant human opioid receptors was mu >> kappa {approx} ORL-1 >> delta. DiPOA showed potent agonist effects in a human mu receptor guanosine 5'-O-(3-[35S]thio)triphosphate functional assay, with an EC50 value of [-7]33 nM and efficacy of [-7]85% {normalized to the mu agonist, [D-Ala2,MePhe4,Gly(ol)5]enkephalin}. Low potency agonist activity was also seen at ORL-1 and kappa receptors. DiPOA bound competitively to the opioid binding site of human mu receptors as demonstrated by a parallel rightward shift in its concentration-response curve in the presence of increasing concentrations of naltraxone. High and sustained ([&ge]5 h) plasma levels for DiPOA were achieved following intraperitoneal administration at 3 and 10 mg/kg; central nervous system penetration, however, was [&le]4% of the plasma concentration, even at levels exceeding 1500 ng/ml. As such, DiPOA represents a systematically available, peripherally restricted small molecule mu opioid agonist that will aid in understanding the role played by mu opioid receptors in the periphery.


http://jpet.aspetjournals.org/cgi/content/abstract/307/3/1148

Elevated glucocorticoid levels are associated with many diseases, including age-related depression, hypertension, Alzheimer's disease, and acquired immunodeficiency syndrome. Cortisol-lowering agents could provide useful complementary therapy for these disorders. We examined the effect of procaine and procaine in a pharmaceutical formulation on adrenal cortical steroid formation. Procaine inhibited dibutyryl cyclic AMP (dbcAMP)-induced corticosteroid synthesis by murine Y1 and human H295R adrenal cells in a dose-dependent manner without
affecting basal steroid formation. Treatment of rats with the procaine-based formulation reduced circulating corticosterone levels. This steroidogenesis-inhibiting activity of procaine was not observed in Leydig cells, suggesting that the effect was specific to adrenocortical cells. In search of the mechanism underlying this inhibitory effect on cAMP-induced corticosteroidogenesis, procaine was found to affect neither the cAMP-dependent protein kinase activity nor key proteins involved in cholesterol transport into mitochondria, cytochrome P450 side chain cleavage enzyme expression, and enzymatic activities associated with cholesterol metabolism to final steroid products. However, procaine reduced in a dose-dependent manner the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) activity and the dbcAMP-induced HMG-CoA reductase mRNA levels by affecting mRNA stability. These data suggest that the inhibitory effect of procaine on cAMP-induced corticosteroid formation is due to the reduced synthesis of cholesterol. This modulatory effect of procaine on HMG-CoA reductase mRNA expression was also seen in dbcAMP-stimulated Hepa1-6 mouse liver hepatoma cells. Taken together, these results suggest that procaine may provide a pharmacological means for the control of hormone-induced HMG-CoA reductase mRNA expression and hypercortisolemia.


http://jpet.aspetjournals.org/cgi/content/abstract/309/2/804

Adenosine has been demonstrated to inhibit gastric acid secretion. In the rat stomach, this inhibitory effect may be mediated indirectly by increasing the release of somatostatin-like immunoreactivity (SLI). Results show that adenosine analogs augmented SLI release in the isolated vascularly perfused rat stomach. The rank order of potency of the analogs in stimulating SLI release was 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680) > 5'-N-ethylcarboxamidoadenosine > 2-chloroadenosine > R-(−)-N6-(2-phenylisopropyl)adenosine > 1-deoxy-1-[[3-iodophenyl]methyl]amino]-9H-purin-9-yl]-N-methyl-(beta)-D-ribofuranuronamide > N6-cyclopentyladenosine (approx) N6-cyclohexyladenosine > S-(+)-N6-(2-phenylisopropyl) adenosine, suggesting the involvement of the A2A receptor. In agreement, 4-(2-[7-amino-2-(2-furyl)]triazolo[2,3-a] [1,3,5]triazin-5-ylamino)ethyl)phenol (ZM 241385), an A2A receptor antagonist, was shown to abolish the adenosine- and CGS 21680-stimulated SLI release. Immunohistochemical studies reveal the presence of A2A receptor immunoreactivity on the gastric plexi and mucosal D-cells, but not on parietal cells and G-cells, suggesting that adenosine may act directly on D-cells or indirectly on the gastric plexi to augment SLI release. The present study also demonstrates that the structure of the mucosal A2A receptor is identical to that in the rat brain, and that alternative splicing of this gene does not occur. A real-time reverse transcription-polymerase chain reaction assay has also been established to quantify the levels of A2A receptor mRNA. Results show that gastric tissues contained significantly lower levels of A2A receptor mRNA compared with the striatum. The lowest level was detected in the mucosa. In conclusion, adenosine may act on A2A receptors to augment SLI release and consequently control gastric acid secretion.


http://jpet.aspetjournals.org/cgi/content/abstract/311/1/180

Adenosine has been shown to inhibit immunoreactive gastrin (IRG) release and to stimulate somatostatin-like immunoreactivity (SLI) release by activating adenosine A1 and A2A receptors, respectively. Since the synthesis and release of gastrin and somatostatin are regulated by the acid secretory state of the stomach, the effect of achlorhydria on A1 and A2A receptor gene
expression and function was examined. Omeprazole-induced achlorhydria was shown to suppress A1 and A2A receptor gene expression in the antrum and corporeal mucosa, but not in the corporeal muscle. Omeprazole treatment produced reciprocal changes in A1 receptor and gastrin gene expression, and parallel changes in A2A receptor and somatostatin gene expression. The localization of A1 and A2A receptors on gastrin-secreting G-cells and somatostatin-secreting D-cells, respectively, suggests that changes in adenosine receptor expression may modulate the synthesis and release of gastrin and somatostatin. Thus, the effect of omeprazole on adenosine receptor-mediated changes in IRG and SLI release was also examined in the vascularly perfused rat stomach. After omeprazole treatment, the A1 receptor-mediated inhibition of IRG and SLI release induced by N6-cyclopentyladenosine (A1 receptor-selective agonist) was not altered, but the A2A receptor-mediated augmentation of SLI release induced by 2-p-(2-carboxyethyl-)phenethylamino-5’-N-ethylcarboxamidoadenosine (A2A-selective agonist) was significantly attenuated. These findings agree well with the corresponding omeprazole-induced decrease in antral A2A receptor mRNA expression. Overall, the present study suggests that adenosine receptor gene expression and function may be altered by omeprazole treatment. Acid-dependent changes in adenosine receptor expression may represent a novel purinergic regulatory feedback mechanism in controlling gastric acid secretion.


http://jpet.aspetjournals.org/cgi/content/abstract/310/2/477

Adenosine has been demonstrated to inhibit gastric acid secretion. In the rat stomach, this inhibitory effect may be mediated indirectly by the inhibition of gastrin release. Results show that the A1 receptor agonist N6-cyclopentyladenosine (CPA) suppressed immunoreactive gastrin (IRG) release in a concentration-dependent manner. CPA significantly inhibited IRG release at 0.001 {micro}M and maximally inhibited IRG release at 1 {micro}M. At concentrations of 0.001 to 0.1 {micro}M, the A2A receptor-selective agonist 2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxamidoadenosine and A3 receptor-selective agonist 1-deoxy-1-[6-[(3-iodophenyl)methyl][l]amino]-9H-purin-9-yl]-N-methyl-[beta]-D-ribofuranuronamide, had no effect on IRG release, suggesting the involvement of A1 receptors. In agreement, the A1 receptor-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine abolished adenosine-induced inhibition of IRG release. Results of immunohistochemistry experiments reveal the presence of A1 receptor immunoreactivity on mucosal G-cells and D-cells, and the gastric plexi, but not parietal cells, suggesting that adenosine may act directly on G-cells or indirectly on the gastric plexi to modulate IRG release. The structure of the mucosal A1 receptor was found to be identical to that in the rat brain. Alternative splicing within the coding region of this receptor did not occur. A real-time reverse transcription-polymerase chain reaction assay was developed to measure gastric A1 receptor gene expression. The highest level of gastric A1 receptor mRNA was found in the corporeal muscle. However, this level was significantly lower in comparison with the striatum. In conclusion, this study shows that adenosine may suppress IRG release, at least in part, by activating A1 receptors localized on G-cells and may consequently result in an inhibition of gastric acid secretion.


http://jpet.aspetjournals.org/cgi/content/abstract/306/2/447

A sensitive quantitative-competitive reverse transcriptase-polymerase chain reaction method was
developed to measure (micro)-opioid receptor (MOR) mRNA expression in SHSY-5Y neuroblastoma cells. Differentiation of SHSY-5Y cells with either retinoic acid (RA) or 12-o-tetradecanoyl-phorbol-13-acetate (TPA) significantly increased MOR mRNA levels. Morphine treatment (10 (micro)M) for 24 h decreased MOR mRNA levels in control, as well as RA- and TPA-differentiated cells. In contrast, chronic exposure to the opioid peptides endomorphin-1 or endomorphin-2 significantly increased MOR mRNA levels in undifferentiated and RA-differentiated cells. An opioid antagonist, naloxone, reversed the morphine and endomorphin-1 and -2 effects on MOR mRNA levels in undifferentiated SHSY-5Y cells, but naloxone had differential reversing effects on the agonists' regulation of MOR mRNA in RA- or TPA-differentiated cells. To investigate whether the changes in MOR mRNA expression paralleled changes in MOR receptor function, intracellular cAMP accumulation in SHSY-5Y cells was measured. After chronic treatment with morphine, forskolin-induced cAMP levels in SHSY-5Y cells were significantly higher than those of untreated control cells. In contrast, forskolin-induced cAMP accumulation levels were lower in cells treated with endomorphin-1 or -2 than in untreated control cells. Together, our studies indicate that the opioid alkaloid morphine and the opioid peptides endomorphin-1 and -2 differentially regulate MOR mRNA expression and MOR function in SHSY-5Y cells.


http://jpet.aspetjournals.org/cgi/content/abstract/310/2/437

The human proton-dependent dipeptide transporter (PEPT1, gene SLC15A1) is important for intestinal absorption of di- and tripeptides and a variety of peptidomimetic compounds. Using a DNA polymorphism discovery panel of 44 ethnically diverse individuals, nine nonsynonymous and four synonymous coding-region single-nucleotide polymorphisms (SNPs) were identified in PEPT1. HeLa cells were transiently transfected with plasmids constructed by site-directed mutagenesis for each of the nine nonsynonymous variants. Quantitative polymerase chain reaction showed that the mRNA transcription level of all of the mutants was comparable with the mRNA transcription level of the reference sequence in transfected HeLa cells. Functional analysis in transiently transfected HeLa cells revealed that all nonsynonymous variants retained similar pH-dependent activity and Kt values for [glycyl-1,2-14C]glycylsarcosine (Gly-Sar) uptake as the reference PEPT1. In addition, a group of seven peptide-like drugs showed inhibitory effect on Gly-Sar uptake by these variants comparable with the reference, suggesting conserved drug recognition. Of the nine nonsynonymous SNPs, a single SNP (P586L) demonstrated significantly reduced transport capacity as evidenced by a much lower Vmax value. This was consistent with lower immunoactive protein level (Western analysis) and lower plasma membrane expression (immunocytochemical analysis). Therefore, Pro586 may have profound effect on PEPT1 translation, degradation, and/or membrane insertion.

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Skeletal muscle has been recognized as an endocrine organ, and muscle cell cultures express several cytokines with potential hormonal effects. Interleukin-8 (IL-8), a chemokine, which induces angiogenesis, is expressed in working muscles; however, the cell source of origin has not been identified. We aimed to elucidate if IL-8 protein is: (1) expressed in contracting muscle fibres and (2) whether there is a release of IL-8 from exercising muscle. Seventeen healthy male volunteers were included in two independent protocols: 3 h of ergometer bicycle exercise at 60% of maximal workload (n = 6) or rest (n = 5), and 3 h of two-legged knee-extensor exercise at 60% of maximal workload (n = 6). Repetitive muscle biopsy samples were obtained from the vastus lateralis in all experiments. A marked increase in IL-8 mRNA was found in muscle biopsy samples obtained after exercise. A marked IL-8 protein expression was demonstrated within the cytoplasm of muscle fibres in biopsy samples obtained in the recovery phase following 3 h of bicycle exercise, and the peak occurred 3-6 h postexercise. A small transient net release of IL-8 from working muscle was found at 1.5 h of knee-extensor exercise. However, the small release of IL-8 from muscle did not result in an increase in the systemic plasma concentration of IL-8, suggesting that muscle-derived IL-8 may play a local role, e.g. in angiogenesis.


RyR3 mRNA was only slightly expressed in purified β-cells. A CICR could also be detected in a limited number of cells in response to glucose. Our data demonstrate, for the first time in living cells, the existence of an atypical CICR that is independent from the IP3R and the RyR. This CICR is prominent in response to a supraphysiological stimulation with high K+, but plays little role in response to glucose in non-obese mouse pancreatic β-cells.


http://jp.physoc.org/cgi/content/abstract/557/1/229

The parvocellular (PC) division of the afferent visual pathway is considered to carry neuronal signals which underlie the red-green dimension of colour vision as well as high-resolution spatial vision. In order to understand the origin of these signals, and the way in which they are combined, the responses of PC cells in dichromatic (red-green colour-blind') and trichromatic marmosets were compared. Visual stimuli included coloured and achromatic gratings, and spatially uniform red and green lights presented at varying temporal phases and frequencies. The sensitivity of PC cells to red-green chromatic modulation was found to depend primarily on the spectral separation between the medium- and long-wavelength-sensitive cone pigments (20 or 7 nm) in the two trichromatic marmoset phenotypes studied. The temporal frequency dependence of chromatic sensitivity was consistent with centre-surround interactions. Some evidence for chromatic selectivity was seen in peripheral PC cells. The receptive field dimensions of parvocellular cells were similar in dichromatic and trichromatic animals, but the achromatic contrast sensitivity of cells was slightly higher (by about 30%) in dichromats than in trichromats. These data support the hypothesis that the primary role of the PC is to transmit high-acuity spatial signals, with red-green opponent signals appearing as an additional response dimension in trichromatic animals.


http://jp.physoc.org/cgi/content/abstract/539/1/107


http://jp.physoc.org/cgi/content/abstract/538/3/773


http://jp.physoc.org/cgi/content/abstract/jphysiol.2005.084574v1

The excitability of smooth muscles is regulated, in part, by background K+ conductances that determine resting membrane potential. However, the K+ conductances so far described in gastrointestinal (GI) muscles are not sufficient to explain the negative resting potentials of these cells. Here we describe expression of two-pore K+ channels of the TASK family in murine small
and large intestinal muscles. TASK-2, cloned from murine intestinal muscles, resulted in a pH sensitive, time-dependent, non-inactivating K+ conductance with slow activation kinetics. A similar conductance was found in native intestinal myocytes using whole-cell patch clamp conditions. The pH-sensitive current was blocked by local anesthetics. Lidocaine, bupivacaine, and acidic pH, depolarized circular muscle cells in intact muscles and decreased amplitude and frequency of slow waves. The effects of lidocaine were not blocked by tetraethylammonium chloride, 4-aminoypyridine, glibenclamide, apamin, or MK-499. However, depolarization by acidic pH was abolished by pre-treatment with lidocaine, suggesting that lidocaine-sensitive K+ channels were responsible for pH-sensitive changes in membrane potential. The kinetics of activation, sensitivity to pH, and pharmacology of the conductance in intestinal myocytes and the expression of TASK-1 and TASK-2 in these cells suggest that the pH-sensitive background conductance is encoded by TASK genes. This conductance appears to contribute significantly to resting potential and may regulate excitability of GI muscles.


http://jp.physoc.org/cgi/content/abstract/555/2/471

Postischaemic acute renal failure (ARF) is influenced by sex. Na+,K+-ATPase (NKA) plays a crucial role in the pathogenesis of postischaemic ARF. We tested the impact of sex on mRNA, protein expression, cellular distribution and enzyme activity of NKA following renal ischaemia-reperfusion (I-R) injury. The left renal pedicle of uninephrectomized female (F) and male (M) Wistar rats was clamped for 55 min followed by 2 h (T2) and 16 h (T16) of reperfusion. Uninephrectomized, sham-operated F and M rats served as controls (n=6 per group). Blood urea nitrogen, serum creatinine and renal histology were evaluated to detect the severity of postischaemic ARF. mRNA expression of NKA {alpha}1 and {beta}1 subunits were detected by RT-PCR. The effect of I-R on cellular distribution was compared by Triton X-100 extraction. Cellular proteins were divided into Triton-insoluble and Triton-soluble fractions and assessed by Western blot. NKA enzyme activity was also determined. After the ischaemic insult blood urea nitrogen and serum creatinine were higher and renal histology showed more rapid progression in M versus F (P<0.05). mRNA expression of the NKA {alpha}1 subunit decreased in I-R groups versus controls, but was higher in F versus M both in control and I-R groups (P<0.05). However, protein levels of the NKA {alpha}1 subunit in total tissue homogenate did not differ in controls, but were higher in F versus M in I-R groups (P<0.05). Triton X-100 extractability was lower in F versus M at T16 (P<0.05). NKA enzyme activity was the same in controls, but was higher in F versus M in I-R groups (T2: 14.9 {+/-} 2.3 versus 9.15 {+/-} 2.21 U; T16: 11.7 {+/-} 4.1 versus 5.65 {+/-} 2.3 U; P<0.05). mRNA and protein expression of the NKA {beta}1 subunit did not differ between F and M in any of the protocol. We concluded that NKA is more protected from the detrimental effects of postischaemic injury in females. Higher mRNA and protein expression of the NKA {alpha}1 subunit and higher enzyme activity might be additional contributing factors to the improved postischaemic renal function of female rats.


http://jp.physoc.org/cgi/content/abstract/561/1/215

The present study was designed to elucidate whether the conduction of vasomotor responses mediated by endothelium-derived hyperpolarizing factor (EDHF) in rat mesenteric arteries is altered during hypertension. Iontophoresed acetylcholine (ACh; 500 ms) caused EDHF-mediated hyperpolarization and vasodilatation at the local site and these responses spread through the
endothelium to remote sites in 12-week-old Wistar-Kyoto rats (WKY). Conducted responses were significantly attenuated in age-matched spontaneously hypertensive rats (SHR) although the rate of decay with distance did not change. Inhibition of inwardly rectifying potassium (Kir) channels (30 \( \mu \)M barium) eliminated the difference between WKY and SHR by attenuating conducted responses in WKY but not SHR. At the local site, barium (30 \( \mu \)M) significantly reduced the duration but not the amplitude of ACh-induced hyperpolarization in WKY only. Barium had no effect when the iontophoretic stimulus was reduced to 350 ms. After blockade of EDHF in SHR, ACh elicited a depolarization which our indirect data suggest spreads along the vessel in the endothelium. Messenger RNA expression of Kir2.0 genes did not differ between the strains nor did the amplitude of K+ -induced hyperpolarization, which was abolished by disruption of the endothelium. Immunohistochemistry revealed a decrease in connexin (Cx)37 but not Cx40 or Cx43 protein in endothelial cells of SHR compared to WKY. Results suggest that conduction of EDHF-mediated responses in WKY, but not in SHR, is facilitated by activation of Kir channels at the site of ACh application and not by differences in endothelial connexin expression. Lack of Kir channel involvement in hypertension may result from reduction in the duration of the hyperpolarization due to the development of ACh-mediated depolarization, rather than to any difference in Kir subunit expression or function.


http://jp.physoc.org/cgi/content/abstract/558/2/561

The M1 and M3 subtypes are the major muscarinic acetylcholine receptors in the salivary gland and M3 is reported to be more abundant. However, despite initial reports of salivation abnormalities in M3-knockout (M3KO) mice, it is still unclear which subtype is functionally relevant in physiological salivation. In the present study, salivary secretory function was examined using mice lacking specific subtype(s) of muscarinic receptor. The carbachol-induced \([Ca^{2+}]_i\) increase was markedly impaired in submandibular gland cells from M3KO mice and completely absent in those from M1/M3KO mice. This demonstrates that M3 and M1 play major and minor roles, respectively, in the cholinergically induced \([Ca^{2+}]_i\) increase. Two-dimensional \(Ca^{2+}\)-imaging analysis revealed the patchy distribution of M1 in submandibular gland acini, in contrast to the ubiquitous distribution of M3. In vivo administration of a high dose of pilocarpine (10 mg kg\(^{-1}\), S.C.) to M3KO mice caused salivation comparable to that in wild-type mice, while no salivation was induced in M1/M3KO mice, indicating that salivation in M3KO mice is caused by an M1-mediated \([Ca^{2+}]_i\) increase. In contrast, a lower dose of pilocarpine (1 mg kg\(^{-1}\), S.C.) failed to induce salivation in M3KO mice, but induced abundant salivation in wild-type mice, indicating that M3-mediated salivation has a lower threshold than M1-mediated salivation. In addition, M3KO mice, but not M1KO mice, had difficulty in eating dry food, as shown by frequent drinking during feeding, suggesting that salivation during eating is mediated by M3 and that M1 plays no practical role in it. These results show that the M3 subtype is essential for parasympathetic control of salivation and a reasonable target for the drug treatment and gene therapy of xerostomia, including Sjogren's syndrome.


http://jp.physoc.org/cgi/content/abstract/546/3/851
Phosphatidylinositol 3-Kinase (PI3-kinase) is activated during and required for hippocampal glutamate receptor-dependent long-term potentiation. It mediates the delivery of AMPA receptors to the neuronal surface. Among the downstream targets of PI3-kinase are three members of the serum- and glucocorticoid-inducible kinase family, SGK1, SGK2 and SGK3. We show here that, in Xenopus oocytes expressing the AMPA subunit GluR1, SGK3 and to a lesser extent SGK2 but not SGK1 increases glutamate-induced currents by increasing the abundance of GluR1 protein in the cell membrane. We further show Sgk3 mRNA expression in the hippocampus by RT-PCR and in situ hybridisation. According to Western blotting the hippocampal abundance of GluR1 is significantly lower in gene-targeted mice lacking SGK3 (Sgk3-/-) than in their wild type littermates (Sgk3+/+). The present observations disclose a novel mechanism in the regulation of GluR1.

OBJECTIVES: The purpose of this study was to profile altered patterns of gene expression that characterize degenerative ascending thoracic aortic aneurysms and to compare these patterns
with those observed for infrarenal abdominal aortic aneurysms. METHODS: Full-thickness aortic wall tissues were obtained during surgical repair of degenerative thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms (n = 4 each), with normal thoracic and abdominal aortas from organ transplant donors used as control preparations. Radiolabeled complementary DNA was prepared for each specimen and hybridized to complementary DNA microarrays, and differential levels of gene expression between aneurysmal and normal aortic tissues at each site were assessed by parametric statistics. RESULTS: Of 1185 genes examined, 112 (9.5%) were differentially expressed (P <.05) between thoracic aortic aneurysms and normal thoracic aorta, with 105 increased and 7 decreased. There were 104 genes (8.8%) differentially expressed between infrarenal abdominal aortic aneurysms and normal abdominal aorta (65 increased and 39 decreased). Quantitative increases in expression for 97 genes were unique to thoracic aortic aneurysms, whereas increases for 61 genes were unique to infrarenal abdominal aortic aneurysms. Although 8 gene products were significantly altered in both thoracic and infrarenal abdominal aortic aneurysms, these changes were directionally concordant for only 4 (matrix metalloproteinase 9/gelatinase B, v-yes-1 oncogene, mitogen-activated protein kinase 9, and intercellular adhesion molecule 1/CD54). Results for 9 genes were independently confirmed by quantitative reverse transcriptase-polymerase chain reaction. CONCLUSIONS: Thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms exhibit distinct patterns of gene expression relative to normal aorta from the same sites, with most alterations being unique to each disease. Degenerative aneurysms arising in different locations are thus characterized by a high degree of molecular heterogeneity, reflecting different pathophysiologic mechanisms.


http://jtcs.ctsnetjournals.org/cgi/content/abstract/123/3/484

Objective: The 5-year survival for patients with surgically resected stage I non-small cell lung cancer is only 60% to 70%, probably because of undetected systemic occult micrometastases. Detection of occult micrometastases in lymph nodes by reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen messenger RNA in non-small cell lung cancer has not been reported. Detection of occult micrometastases by standard reverse-transcriptase polymerase chain reaction provides only yes or no answers about their presence, whereas quantitative real-time reverse-transcriptase polymerase chain reaction permits reproducible quantitation of target molecules. This study evaluated the ability of quantitative reverse-transcriptase polymerase chain reaction to quantitate lymph node occult metastases with carcinoembryonic antigen messenger RNA as a tumor marker. Methods: Standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen messenger RNA were performed on 232 lymph nodes from 53 patients with stage I disease (node negative according to histologic examination). Quantitative reverse-transcriptase polymerase chain reaction determined carcinoembryonic antigen messenger RNA quantity by detecting fluorescence increase at a threshold polymerase chain reaction cycle. Threshold polymerase chain reaction cycle values were correlated with standard curves created from serially diluted carcinoembryonic antigen-positive HTB-174 tumor cells to estimate the number of micrometastatic tumor cells in a lymph node. Results: Detection rates of occult metastases were similar for standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction at 38 of 232 (16.4 %) and 59 of 232 (25.4 %), respectively. Upstaging rates among 53 cases of stage I non-small cell lung cancer were also similar for standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction at 23 of 53 (43.4 %) and 30 of 53 (56.6%), respectively. Comparison of positive lymph node stations according to quantitative reverse-transcriptase polymerase chain reaction (threshold polymerase chain reaction cycle <45) with
HTB-174 tumor cell standard curves yielded estimates of metastatic tumor cell burden of 1.07 x 10^3 to 3.24 x 10^5 cells per lymph node station (median 7190 tumor cells per lymph node station).

Conclusions: Standard and quantitative real-time reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen detected occult metastases in patients with stage I non-small cell lung cancer at similar rates; both upstaged about 50% of cases. Quantitative reverse-transcriptase polymerase chain reaction allows estimation of the number of metastatic cells per lymph node, however, which potentially allows greater precision in predicting recurrence risk.


http://jtc.s.cnsjournals.org/cgi/content/abstract/125/2/246

Objective: This study was undertaken to investigate the role of the angiogenic factors vascular endothelial growth factor and basic fibroblast growth factor in the development and progression of Barrett esophagus and adenocarcinomas of the esophagus and gastroesophageal junction.

Methods: Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels, relative to the control gene encoding (beta)-actin, were measured by using a quantitative reverse transcription-polymerase chain reaction method (ABI 7700 Sequence Detector system) in specimens of Barrett intestinal metaplasia (n = 16), dysplasia (n = 11), adenocarcinoma (n = 15), and matching normal squamous esophageal tissues (n = 35). Vascular endothelial growth factor and basic fibroblast growth factor protein expression and CD31+ microvessel density were assessed by means of immunohistochemistry in 25 tissue sections that included representative areas for each of these Barrett stages. Results: Expression levels were significantly increased in adenocarcinoma compared with either normal squamous mucosa (P < .0001 for both genes) or intestinal metaplasia (vascular endothelial growth factor, P = .002; basic fibroblast growth factor, P < .0001). Vascular endothelial growth factor levels were also significantly higher in cancer tissues compared with dysplasia tissues (P = .024, Mann-Whitney U test). Basic fibroblast growth factor expression was also significantly increased in Barrett dysplastic mucosa compared with in intestinal metaplasia or normal esophageal mucosa. Microvessel density was generally higher in adenocarcinoma compared with in preneoplastic Barrett tissues. The pattern of vascular endothelial growth factor and basic fibroblast growth factor protein expression was similar to the messenger RNA expression pattern, with the exception that mucin-containing goblet cells stained intensely for vascular endothelial growth factor and only weak vascular endothelial growth factor staining was present in some adenocarcinomas. Conclusions: Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels are significantly upregulated in esophageal and gastroesophageal junction adenocarcinomas, suggesting a role for these angiogenic factors in the development of these cancers. Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels are also increased in some Barrett esophagus tissues, with this increase occurring at an earlier stage for basic fibroblast growth factor than for vascular endothelial growth factor. Basic fibroblast growth factor protein expression pattern is similar to the messenger RNA expression pattern, but unlike the messenger RNA findings, vascular endothelial growth factor protein expression is strongest in goblet cells.

Lungs of 102 roe deer (Capreolus capreolus), 136 moose (Alces alces), 68 fallow deer (Dama dama), and six red deer (Cervus elaphus) were examined during hunting seasons from 16 September 1997 to 1 March 2000. The aim was to determine the species composition and prevalence of Dictyocaulus lungworms in these hosts in Sweden. Worms were identified following polymerase chain reaction (PCR) amplification of the internal transcribed spacer of ribosomal DNA (ITS2), followed by hybridization with four species-specific oligonucleotides. In addition, 50 lungworms from five reindeer (Rangifer tarandus) from Norway were similarly analyzed. A total of 399 worms were recovered and analyzed representing a range of 29-128 worms per host species. All specimens from roe deer were identified as Dictyocaulus capreolus, whereas those from red deer and reindeer were identical with D. eckerti. From moose, 73 (81.1%) of the worms were identified as D. capreolus whereas 17 (18.9%) were D. eckerti. The ITS2 sequence of fallow deer lungworms differed significantly when compared with the ITS2 of D. viviparus, D. capreolus, and D. eckerti. This indicated that fallow deer in Sweden may be infected with a new genotype of Dictyocaulus spp. Consequently, a specific probe designed for the ITS2 from this Dictyocaulus sp. hybridized exclusively with samples from lungworms of fallow deer. Interestingly, no D. viviparus were found in any of these hosts. The prevalence of infection in each host was as follows: D. capreolus in roe deer (14.7%) and moose (10.6%); D. eckerti in moose (0.7%) and red deer (33.3%); and Dictyocaulus sp. in fallow deer (10.3%). Regardless of lungworm species, the overall prevalence of Dictyocaulus spp. in these hosts was 12.2%. Prevalence between male and female animals and among the different age groups did not differ significantly. Finally an enzyme linked immunosorbent assay (ELISA) specific for patent D. viviparus infection in cattle was utilized to analyze lung tissue fluids from infected animals. All samples from roe deer, red deer, and fallow deer were negative in the ELISA. However, three out of twelve (25%) samples from moose and 17 of 40 (43%) samples from cattle were positive. This indicated that moose anti-D. capreolus antibodies recognized the D. viviparus antigen and that anti-cattle immunoglobulin cross-reacted with moose antibodies.


During the quarantine examination of four Pallas's cats (Otocolobus manul) imported from Mongolia in October and December 2000, intraerythrocytic piroplasms were detected on Wright-Giemsa stained blood films that were morphologically indistinguishable from other small piroplasms of felids. Further characterization of this unknown organism via polymerase chain reaction amplification, sequencing of a portion of the 18S nuclear small subunit rRNA gene, and comparisons with orthologous sequences from other piroplasms, revealed similarity to Cytauxzoon felis. This is the first report of naturally occurring erythoparasitemia in Pallas's cats and the first documented case of naturally occurring piroplasm infections in a free-ranging felid from Mongolia.

In two studies conducted from October 1999 to March 2000 and December 2000 to April 2001, adult black bears (Ursus americanus) were orally inoculated with 1.4-3.1x10^{10} colony-forming units (CFU) of Brucella abortus strain RB51 (SRB51, n=12) or 2 ml of 0.15 M NaCl solution (saline, n=11). We did not detect a difference (P>0.05) in antibody titers to SRB51 in serum obtained before vaccination, at 8 wk after vaccination, or at necropsy at 21 or 23 wk after vaccination between SRB51-vaccinated and nonvaccinated bears. The SRB51 vaccine strain was recovered from tissues obtained at necropsy from one of six SRB51-vaccinated bears in study 1, but none of the six SRB51-vaccinated bears in study 2. Vaccination of black bears with SRB51 did not appear to influence (P>0.05) reproductive performance.


In the 1980s, alligators (Alligator mississippiensis) of Lake Apopka (Florida, USA) underwent a population decline associated with decreased egg viability, effects that have been associated with endocrine-disrupting, persistent organochlorine pesticides. It is currently unknown whether the decreased egg viability is due to fertilization failure or early embryonic death. Therefore, we conducted a preliminary study to evaluate the use of microsatellite DNA loci to determine the fertilization status of nonviable eggs. Using microsatellite analysis, we compared genotypes from blasto-disks and embryos with the genotypes from females trapped at the nest. Four of five nonviable egg samples tested yielded evidence of fertilization. No evidence of unfertilized eggs was obtained, but amplifiable DNA could not be obtained from one entirely nonviable clutch. Thus, we demonstrate that early embryonic mortality in alligators can be detected by microsatellite analysis, but also suggest substantial effort is needed to improve the recovery of DNA and amplification of alligator microsatellite loci.


Organ samples from free-living raptors from the federal states of Berlin and Brandenburg in eastern Germany were tested for Newcastle disease virus (NDV; n = 331) and Chlamydia psittaci (n = 39) by polymerase chain reaction (PCR). In 18 individuals NDV nucleic acids were detected. These samples originated from barn owls (Tyto alba; n = 15, 28%), tawny owl (Strix aluco; n = 1, 5%), common buzzard (Buteo buteo; n = 1, 1%), and European kestrel (Falco tinnunculus; n = 1, 4%). In 29 (74%) of 39 samples C. psittaci was detected. Chlamydia psittaci is common in free-living birds of prey in the investigated area.

Paratuberculosis is endemic in domestic and wild ruminants worldwide. We designed the following study to compare host immune responses and pathologic changes in beef calves and bison calves after challenge with either a cattle or bison (Bison bison) strain of Mycobacterium avium subsp. paratuberculosis. In the first part of the study, six bison and six beef calves were orally inoculated with a cattle isolate of M. avium subsp. paratuberculosis over a 2 wk period. In the second part, an additional six bison and six beef calves were similarly inoculated with a bison strain of M. avium subsp. paratuberculosis. Throughout each of the studies, blood and fecal samples were taken monthly for a 6 mo infection period. Tissue samples were obtained at necropsy for culture and histopathologic analyses. Results from this study demonstrated that bison calves were more susceptible to tissue colonization than beef calves after challenge with the cattle isolate and, conversely, that beef calves were more susceptible to the bison strain of M. avium subsp. paratuberculosis. Although lesions were minimal they were most apparent in the jejunum and distal ileum. Interferon-gamma (IFN-gamma) responses were noted in some calves by 1 mo postinoculation and were sustained longer in beef calves after challenge with the bison isolate. Antibody was not detected in either beef or bison calves during the 6 mo infection period. These results indicate that the host response to strains of M. avium subsp. paratuberculosis may differ between ruminant species.


http://jama.ama-assn.org/cgi/content/abstract/287/13/1671

Context Development of new biomarkers for ovarian cancer is needed for early detection and disease monitoring. Analyses involving complementary DNA (cDNA) microarray data can be used to identify up-regulated genes in cancer cells, whose products may then be further validated as potential biomarkers. Objective To describe validation studies of an up-regulated gene known as osteopontin, previously identified using a cDNA microarray system. Design, Setting, and Participants Experimental and cross-sectional studies were conducted involving ovarian cancer and healthy human ovarian surface epithelial cell lines and cultures, archival paraffin-embedded ovarian tissue collected between June 1992 and June 2001, and fresh tissue and preoperative plasma from 144 patients evaluated for a pelvic mass between June 1992 and June 2001 in gynecologic oncology services at 2 US academic institutions. Plasma samples from 107 women selected from an epidemiologic study of ovarian cancer initiated between May 1992 and March 1997 were used as healthy controls. Main Outcome Measures Relative messenger RNA expression in cancer cells and fresh ovarian tissue, measured by real-time polymerase chain reaction as 2-{Delta}{Delta}CT(a quantitative value representing the amount of osteopontin expression); osteopontin production, localized and scored in ovarian healthy and tumor tissue with immunohistochemical studies; and amount of osteopontin in patient vs control plasma, measured using an enzyme-linked immunoassay. Results The geometric mean for 2-\{Delta}\{Delta\}CTfor osteopontin expression in 5 healthy ovarian epithelial cell cultures was 4.1 compared with 270.4 in 14 ovarian cancer cell lines (P =.03). The geometric mean 2-\{Delta}\{Delta\}CTfor osteopontin expression in tissue from 2 healthy ovarian epithelial samples was 9.0 compared with 164.0 in 27 microdissected ovarian tumor tissue samples (P =.06). Immunolocalization of osteopontin showed that tissue samples from 61 patients with invasive ovarian cancer and 29 patients with borderline ovarian tumors expressed higher levels of osteopontin than tissue samples from 6 patients with benign tumors and samples of healthy ovarian epithelium from 3 patients (P =.03). Osteopontin levels in plasma were significantly higher
(P<.001) in 51 patients with epithelial ovarian cancer (486.5 ng/mL) compared with those of 107 healthy controls (147.1 ng/mL), 46 patients with benign ovarian disease (254.4 ng/mL), and 47 patients with other gynecologic cancers (260.9 ng/mL). Conclusions Our findings provide evidence for an association between levels of a biomarker, osteopontin, and ovarian cancer and suggest that future research assessing its clinical usefulness would be worthwhile.


http://jama.ama-assn.org/cgi/content/abstract/288/14/1749

Context Human papillomavirus (HPV) DNA testing of women having Papanicolaou (Pap) smears showing atypical squamous cells of undetermined significance (ASCUS) has clinical usefulness. Whether HPV DNA testing alone is useful in primary screening remains to be determined.

Objective To determine the accuracy of HPV DNA testing for detecting cervical intraepithelial neoplasia (CIN) grade 3 or cancer (the criterion standard). Design, Setting, and Participants Between December 1997 and October 2000, 4075 women who attended Planned Parenthood clinics in Washington State were screened simultaneously using thin-layer Pap and HPV DNA testing by a polymerase chain reaction (PCR)-based method and by a liquid-based RNA-DNA hybridization capture with signal amplification assay (signal amplification). Women who were positive for high-risk HPV types, or had Pap results of ASCUS or higher, were considered to have positive screening test results and were referred for colposcopy and biopsy. Additionally, a random sample of women with negative screening test results was referred for colposcopy. Based on individual and combined thin-layer Pap, HPV PCR, and HPV signal amplification test results from the screening and the colposcopy visits, 7 colposcopy triage strategies were defined and evaluated. Main Outcome Measure Sensitivity and specificity for detecting cervical lesions graded CIN 3 or higher for each of the 7 triage strategies. Results The estimated prevalence of CIN 3 or higher was 3.2%. The sensitivity (95% confidence interval) of thin-layer Pap (with a result of [≥]ASCUS) for identifying women with CIN 3 or higher was only 61.3% (48.5%-70.9%) compared with 88.2% (78.9%-93.8%) for HPV testing by PCR and 90.8% (83.1%-95.8%) by signal amplification. Differences in specificities were also observed: 82.4% (81.8%-83.1%) for thin-layer Pap (with a result of [≥]ASCUS), 78.8% (77.9%-79.7%) for PCR, and 72.6% (69.4%-75.0%) for signal amplification. Compared with referral for colposcopy of all women with ASCUS or higher, signal amplification testing of women with ASCUS and referral of those with a positive result was about as sensitive (61.3% vs 60.3%, respectively) and significantly more specific (82.4% vs 88.9%, respectively). The strategy requiring repeat positive PCR tests on 2 visits had a sensitivity of 84.2% (75.3%-91.0%) and a specificity of 86.2% (85.1%-87.3%). All tests were more specific and less sensitive in older ([≥]30 years) vs younger women.

Conclusions Testing for HPV has higher sensitivity but lower specificity than thin-layer Pap screening. In some settings, particularly where screening intervals are long or haphazard, screening for HPV DNA may be a reasonable alternative to cytology-based screening of reproductive-age women.

Japanese Journal of Ophthalmology (1)


http://www.sciencedirect.com/science/article/B6VJ7-48H218P-8/2/7516af1e1363e818e1aa3558586847bb

**Purpose:** To investigate cytokine mRNA expression during the inflammatory process induced in the contralateral eyes by unilocular inoculation of herpes simplex virus type 1 (HSV-1) via the anterior chamber. **Methods:** BALB/c mice were inoculated in the anterior chamber with 5 x 10^4 plaque-forming units of HSV-1 (KOS). mRNA was extracted from the inflamed posterior segments of the uninoculated eyes at 0 (control), 9, 11, 14, and 21 days postinoculation (p.i.). Reverse transcription-polymerase chain reaction was performed for semiquantitative analysis of mRNA expression of interleukin (IL)-1β, IL-2, IL-4, IL-10, IL-12p35, IL-12p40, interferon (IFN)γ, tumor necrosis factor (TNF)α, transforming growth factor (TGF)β, and induced nitric oxide synthase (iNOS). **Results:** Peak mRNA expression of iNOS was observed at day 14 p.i. The time profiles of mRNA expression for IL-1β, IL-2, IL-4, IL-10, IFN-γ, TNFα, TGFβ, and IL-12p35 and IL-12p40 demonstrated a reverse pattern. **Conclusions:** The kinetics of the analyzed cytokines synchronized with the clinicopathological activity of the experimental murine HSV-1 retinitis. The immunosuppressive cytokines TGFβ and IL-10 demonstrated different peaks of mRNA expressions suggesting that the down-regulation phase of the inflammatory process was controlled by several factors working at different phases.

**Journal of Affective Disorders** **(2)**


http://www.sciencedirect.com/science/article/B6T2X-46G3SNW-11/2/2c42dea4e95ee1494615917938b6122

**Introduction:** Several lines of research point to a possible overlap between seasonal affective disorder (SAD) and attention deficit hyperactivity disorder (ADHD), particularly in females. There is also emerging evidence that variation of the 5-HT2A receptor gene (HTR2A) contributes to both SAD and ADHD. The current study investigated whether variation in HTR2A was associated with symptoms of childhood ADHD in adult women with SAD. **Method:** Sixty-six women with SAD were administered the Wender-Utah Rating Scale (WURS), which retrospectively assesses childhood ADHD, as part of an ongoing genetic study of SAD. WURS scores were compared across the three genotypic groups defined by the T102C polymorphism of HTR2A. **Results:** Analysis of variance indicated a significant difference in mean 25-item WURS scores across the three genotypic groups (p=0.035). Post-hoc tests revealed that the C/C genotypic group had a significantly higher mean score than both the T/T group and T/C group. Based on previously established WURS criteria, 38% of subjects with the C/C genotype, and none with the T/T genotype, had scores consistent with childhood ADHD. **Limitations:** The current sample size is small, and childhood ADHD diagnoses were based on retrospective recall. **Conclusion:** These preliminary results suggest a possible association between variation in HTR2A, childhood ADHD, and the later development of SAD in women.
The recently cloned dopamine D3 receptor (DRD3) gene is of potential relevance to the aetiology of bipolar disorder because of an almost exclusive expression in limbic tissue, the region of the brain putatively responsible for control of emotion. We therefore aimed to determine whether bipolar disorder in nine pedigrees (with 171 members) was linked to this receptor gene, which has been mapped to chromosomal region 3q 13.3. Linkage of bipolar disorder and recurrent depression to the DRD3 gene was tested using a series of autosomal dominant and recessive models with varying penetrance levels. Additionally, linkage was examined using a series of levels of definitions of affective illness (ranging from bipolar I alone to all affective disorders). Close linkage to the DRD3 gene was strongly excluded using each model and definition, and these conclusions persisted when a wide range of rates of 'sporadic' (non-genetic) presentations of illness were incorporated in the analysis.


Background [alpha] and [beta]-Tryptase levels in serum are clinical tools for the evaluation of systemic anaphylaxis and systemic mastocytosis. Basophils and mast cells are known to produce these proteins. Objective The current study examines the effect of the [alpha],[beta]-tryptase genotype on basophil tryptase levels and the type of tryptase stored in these cells. Methods Tryptase extracted from purified peripheral blood basophils from 20 subjects was examined by using ELISAs measuring mature and total tryptase and by using an enzymatic assay with tosyl-Gly-Pro-Lys-p-nitroanilide. Tryptase genotypes (4:0, 3:1, and 2:2 [beta]/[alpha] ratios) were assessed by using a hot-stop PCR technique with [alpha],[beta]-tryptase-specific primers. Total [alpha],[beta]-tryptase mRNA was measured by means of competitive RT-PCR, and ratios of [alpha] to [beta]-tryptase mRNA were measured by means of hot-stop RT-PCR. Results Tryptase in all but one of the basophil preparations was mature and enzymatically active. Tryptase quantities in basophils were less than 1% of those in tissue mast cells. Tryptase genotypes ([beta]/[alpha]) among the 20 donors were 4:0 in 7, 3:1 in 7, and 2:2 in 6. Tryptase protein and mRNA levels per basophil were not affected by the tryptase genotype. Conclusion Basophils from healthy subjects contain modest amounts of mature and enzymatically active tryptase unaffected by the tryptase genotype.


http://www.sciencedirect.com/science/article/B6WH4-4CS46C4-19/2/6287b60b49ecc666c1442e4fba184209

BackgroundThe total level of [alpha]-tryptase and [ss]-tryptase in serum or plasma is used as a clinical indicator of the mast cell burden.ObjectiveThe effect of the tryptase haplotype and of sex on the total tryptase level of healthy individuals was determined.MethodsA novel hot-stop PCR technique was used to determine the tryptase genotype, and a standard fluoroenzyme immunoassay was used to measure total plasma tryptase levels in 106 healthy subjects. Mx modeling and the QTL association routine of Mendel 5.0 were used to analyze the data.ResultsTryptase haplotypes exhibit a 1 ([ss][alpha]/[ss][alpha]):2 ([ss][ss]/[ss][alpha]):1 ([ss][ss]/[ss][ss]) distribution, monomorphic for [ss] at 1 position and allelic for [ss] and [alpha] at the other position. The [ss][alpha] haplotype has a frequency of 0.49. The [ss][alpha] haplotype increases total tryptase levels by 0.5 ng/mL from the overall mean, whereas female sex increases the level by 0.2 ng/mL from the mean.ConclusionThe tryptase haplotype and sex each have a statistically significant effect on the total plasma tryptase level of healthy subjects.


http://www.sciencedirect.com/science/article/B6WH4-4C5YH61-1X/2/4ea2ecd85a3f91408ecd5f59d5083b0e


http://www.sciencedirect.com/science/article/B6WH4-4C5YH61-1S/2/bb20ef226e632a3e772d1bb264dff411

BackgroundAtopic dermatitis (AD) is a chronic inflammatory skin disease involving colonization by superantigen (SAg)-secreting Staphylococcus aureus. CD4+CD25+ T regulatory (Treg) cells are thought to play an important role in controlling inflammatory responses.ObjectiveIn this study we examined whether Treg cells might be deficient in patients with AD.MethodsCD4+CD25+ and CD4+CD25- T cells were isolated from PBMCs by using immunomagnetic beads. Cells were cultured with anti-CD3 or SAg, staphylococcal enterotoxin B (SEB), for 72 hours. Proliferation was measured by means of tritiated thymidine incorporation. CD4, CD8, CD25, and cutaneous lymphocyte-associated antigen expression on PBMCs was assessed by means of flow cytometry. RNA was extracted from isolated subsets of T cells, and the results of real-time PCR for FoxP3 mRNA were determined.ResultsSurprisingly, CD4+CD25+ T cells were significantly (P + in skin-homing, CD4+, cutaneous lymphocyte-associated antigen-positive T cells than asthmatic and nonatopic subjects, with values of 35.95% versus 22.44% versus 23.03%, respectively (P +CD25+ cells expressed FoxP3, whereas CD4+CD25- T cells and CD4- cells did not. Consistent with known properties of Treg cells, CD4+CD25+ cells were anergic to anti-CD3 stimulation. When CD4+CD25+ cells from each study group were mixed with CD4+CD25- cells, proliferative
responses were equally suppressed after anti-CD3 stimulation. In contrast, after SEB stimulation, CD4+CD25+ cells were no longer anergic. Furthermore, when CD4+CD25+ cells were mixed with CD4+CD25- cells and stimulated with SEB, the suppressive function of Treg cells was reversed. Conclusion Patients with AD have significantly increased numbers of peripheral blood Treg cells with normal immunosuppressive activity. However, after SAg stimulation, Treg cells lose their immunosuppressive activity. These data suggest a novel mechanism by which SAg could augment T-cell activation in patients with AD.


http://www.sciencedirect.com/science/article/B6WH4-49W6061-1B/2/aed95e490b7cf57540a02596335ac880

Background Early in life, natural exposure to microbial components (eg, endotoxin) may mitigate allergy and asthma development in childhood. Bacterial DNA is a potent stimulus for the innate immune system; its immune stimulatory potential in dust is unknown. Objectives We sought to quantify bacterial DNA and endotoxin content in dust from urban homes, rural homes, farm homes, and farm barns and to determine if dust DNA is immune-stimulatory. Methods Total DNA, bacterial DNA, and endotoxin were measured in 32 dust samples. To measure bacterial DNA content, a quantitative polymerase chain reaction assay specific for bacterial ribosomal DNA was developed. Peripheral blood mononuclear cells from 5 adults were stimulated with endotoxin-free dust DNA with/without lipopolysaccharide (LPS) from selected dust samples. IL-12p40, IL-10, and tumor necrosis factor-[alpha] were measured in cell supernatants by enzyme-linked immunosorbent assay. Results Bacterial DNA in dust correlated with endotoxin (r = 0.56, P r = 0.51, P = .003). The highest bacterial DNA levels were measured in farm barns (mean, 22.1 [mu]g/g dust; range, 1.3 to 56.2), followed by rural homes (6.3 [mu]g/g; 0.2 to 20), farm homes (2.2 [mu]g/g; 0.1 to 9.1), and urban homes (0.6 [mu]g/g; 0.1 to 1.2). Farm barn DNA significantly potentiated (P Conclusions Endotoxin is a marker for bacterial DNA, which is also higher in locales of lower asthma and allergy prevalence. DNA from farm barn dust augments the immune modulatory effects of endotoxin and may combine with exposure to other such naturally occurring microbial components to mitigate allergy and asthma development.


http://www.sciencedirect.com/science/article/B6WH4-4BMC07G-1C/2/c3d030c51782d264d6825b47c3fe86c

Background TH2 cytokines play a central role in the pathogenesis of allergic asthma. We previously showed that the "antiasthma" Chinese herbal formula MSSM-002 exhibited therapeutic effects on established allergic airway responses in a murine model of allergic asthma. However, the mechanisms underlying these effects are largely unknown. Objective The objective of this study was to determine whether and how MSSM-002 modulates an established TH2 response and whether the actions of MSSM-002 on TH2 cell differs from corticosteroids. Methods TH2 polarized splenocytes (TH2-SPCs) from mice with antigen-induced airway hyperresponsiveness and TH2 cloned cells, D10 G4.1 (D10), were cultured in the presence or absence of antigen with or without MSSM-002 and dexamethasone, and the proliferative responses and cytokine profiles were determined. Apoptosis and TH2 transcription factor GATA-3 expression and binding to IL-4 gene promoter and VA enhancer in MSSM-002-treated D10 cells were also determined. Results MSSM-002 significantly decreased antigen-induced proliferation and IL-4 and IL-5 production but increased IFN-[gamma] production by TH2-SPCs, whereas dexamethasone...
suppressed IFN-γ as well as IL-4 and IL-5. Anti-IL-12 antibody, although abrogating MSSM-002 induction of IFN-γ, had no significant effect on MSSM-002 suppression of IL-4 and IL-5 secretion. MSSM-002 also suppressed TH2 cytokine secretion by D10 cells, and in contrast to dexamethasone, MSSM-002 did not induce apoptosis of D10 cells. MSSM-002 markedly suppressed GATA-3 mRNA and protein expression and the binding to IL-4 gene promoter and VA enhancer in D10 cells. Conclusion MSSM-002, in contrast to the overall suppression of T cells by dexamethasone, exhibits immunomodulatory actions on TH2 cells caused, at least partially, by downregulation of GATA-3.

**Journal of Archaeological Science** (3)


http://www.sciencedirect.com/science/article/B6WH8-4BH64WX-5/2/bea0b91b551e6b66e86de4613695e3e6

A fully articulated dog skeleton excavated from a 16th-century Neutral Iroquoian site in Ontario, Canada displays a distinctive osteological condition known as hypertrophic osteopathy (HPO). Ancient DNA (aDNA) analysis of the dog has isolated Mycobacterium tuberculosis complex DNA, linking the secondary condition of HPO to tuberculosis (TB) and representing the oldest known case of TB yet to be discovered in the domestic dog. We emphasize that dogs should be considered as potential reservoirs of TB into the Americas.


http://www.sciencedirect.com/science/article/B6WH8-4B53YBF-1/2/3bdc2f1e331ce325510afaa5d127bef6

This paper reports on the development and application of methods for using DNA analysis for species identification of archaeological salmon bone. Short fragments (less than 300 bp) of mitochondrial DNA from the control region (D-loop) and cytochrome B (CytB) gene were targeted for amplification using the polymerase chain reaction (PCR) technique. The method was used on more than 20 salmon bone samples (dated 7000 to 2000 BP) from the site of Namu on the central coast of British Columbia. Four species: coho, sockeye, pink and chum salmon were identified from the samples. The results are considered valid since systematic contaminations were not detected, multiple species and multiple DNA haplotypes of the same species were identified from the same set of bone samples, and the identified species are consistent with those inferred from other lines of evidence. The results demonstrate the applicability of the ancient DNA technique to species identification of even single salmon vertebrae from archaeological sites in the Pacific Northwest of North America.

Ancient DNA analysis was carried out on 20 archaeological rabbit remains from an early Pueblo II period site in Colorado (circa 1000 A.D.) to explore the possibility of obtaining accurate rabbit genus and species identifications. The presence of abundant rabbit remains at archaeological sites in the American Southwest indicates the importance of rabbit species in the subsistence economy and ritual activities of early aboriginal populations. The study of these remains is hindered by the difficulty of accurate identification due to the fragmentary nature of the bones and the lack of genus- and species-specific morphological features. A short cytochrome b gene fragment was amplified and sequenced to produce a genetic profile for each bone sample. At the genus level, the DNA identifications were consistent with those based on the analysis of mandible morphology for the majority of specimens. When compared to species-specific reference DNA sequences, Lepus americanus and Lepus californicus samples were easily identified. Identification of an unexpected L. americanus (snowshoe hare) from the remains provided new information concerning hunting ranges or exchange between groups in the region. Sylvilagus nuttallii and Sylvilagus auduboni, however, could not be confidently differentiated at this point due to the difficulty in obtaining accurate species-specific reference sequences. The inability to obtain such reference sequences can be a serious problem for DNA species identification of non-domestic animals that lack population-level genetic data and have few sequences available in GenBank. The lack of the DNA data increases the possibility that inappropriate reference sequences could be applied, resulting in false species identification even when authentic DNA is retrieved and amplified from ancient remains.

Interleukin-18 (IL-18) is a potent proinflammatory cytokine which is strongly associated with the development of diabetes in NOD mice. To test the putative involvement of IL-18 gene polymorphism in predisposition to human type 1 diabetes, the SNPs at position -607 (C/A) and -137 (G/C) in the promoter region of IL-18 gene were analyzed by sequence-specific PCR in 116 patients with type 1 diabetes and 114 normal controls. A linkage disequilibrium found only three of the four possible haplotypes defined by these SNPs. The distribution of the IL-18 gene genotypes at position -607 was significantly different between patients with type 1 diabetes and normal controls (P=0.023). Furthermore, there was a significant increase in haplotype 1 (-607C/-137G) in the patients compared with controls (P=0.006). The association study of the susceptible CTLA-4 genotype (GG at nucleotide position 49 in exon 1) or HLA-DR4-DQB1*0401 and type 1 diabetes showed that the predisposing IL-18 gene haplotype modulates the risk on CTLA-4 GG genotype, but not on HLA-DR4-DQB1*0401 haplotype. Among subjects carrying the CTLA-4 GG genotype, the frequency of IL-18 haplotype 1 in patients with type 1 diabetes was significantly higher than that in controls (91% vs. 71%, P=0.012). However, IL-18 haplotype 1 was not frequent in patients who do not exhibit the CTLA-4 high-risk genotype. These results suggest that the IL-18 gene polymorphism is associated with a type 1 diabetes susceptibility, and there might be a gene-gene interaction between IL-18 gene with susceptible CTLA-4 gene.

http://www.sciencedirect.com/science/article/B6WHC-494HM8F-1/2/69e6bdb8a3967fb3ab540909ecccc04

Evaluation of genes regulated differentially is essential for the development of therapeutic approaches in multifactorial diseases. To characterize gene expression profiles in multifactorial inflammatory and malignant diseases such as rheumatoid arthritis (RA) or colon adenoma (CA), RNA arbitrarily primed PCR (RAP-PCR) combined with cDNA array hybridization were performed and evaluated using an array-specific software. RNA of synovial fibroblasts from patients with RA and osteoarthritis (OA), and laser microdissected normal and colon adenoma tissue was used. RAP-PCR reactions were hybridized to cDNA array membranes. Arrays were analyzed by phosphor imaging, and the AtlasImage(TM) 2.0 software with different normalization settings. The AtlasImage(TM) 2.0 software was a useful tool to evaluate differentially expressed genes. However, software settings were needed to be optimized for every experimental approach and should be used without changes for all experiments. To compare RA vs. OA synovial fibroblasts and normal vs. CA expression patterns, global normalization using the sum method is recommended.


http://www.sciencedirect.com/science/article/B6WHC-4CWBJRN-2/2/21172f2666ae69962a7442d7a58395a8

Celiac disease (CD) is a complex genetic disorder characterized by gluten intolerance. The Th1 immune response, with a key position for interferon gamma (IFN-[gamma]), is an important determinant of intestinal remodeling in CD. We aimed at further ascertaining the role of IFN-[gamma], either as a genetic factor in the etiology, or as a facilitator of disease initiation/progression. Duodenal biopsies were sampled across distinct histopathological stages of the disease, including refractory CD (RCD), and used to determine IFN-[gamma] gene (IFNG) expression by real-time RT-PCR. INFG expression correlated with the extent of tissue restructuring, reaching a 240-fold higher expression in total villous atrophy compared to healthy tissue. CD and RCD patients with similar lesions had comparable expression levels. Interestingly, patients in complete remission still had 7.6-fold residual over-expression. An INFG marker was tested in three cohorts of Dutch patients for both genetic linkage and association. Linkage analysis yielded no significant scores for IFNG or its flanking markers. In addition, IFNG allele frequencies were not differently distributed between cases and controls. Likewise, all alleles were randomly transmitted to affected children in parents-case trios. There is no evidence for IFNG as a predisposing gene in CD, despite its enhanced expression in patients in complete remission.


http://www.sciencedirect.com/science/article/B6WHC-4DB57WR-D/2/7caf4be3e45267f0477464ad143bd923
Autoreactive T cells specific for myelin basic protein (MBP) are part of the normal T cell repertoire and are present both in patients with multiple sclerosis (MS) and healthy individuals. There is evidence suggesting in vivo activation and persistent clonal expansion of MBP-reactive T cells in MS. This study was undertaken to investigate the potential role of bacterial superantigens (SA) in the activation of MBP-reactive T cells. Twenty-seven MBP-reactive T cell clones generated from 10 MS patients and one normal individual were examined for reactivity to SA, in association with their T cell receptor V[beta] gene usage. The majority of the clones responded to at least one of the SA tested, staphylococcal enterotoxins (SEA and SEB) and toxic shock syndrome toxin-1 (TSST-1). The clones reactive to SEA and SEB expressed various V[beta] genes while T cell reactivity to TSST-1 correlated with the V[beta]2 expression. Furthermore, circulating MBP-reactive T cells could be expanded from lymphocyte cultures primarily exposed to respective SA in more than 50% of MS patients and normal individuals tested. However, activation and expansion of circulating MBP-reactive T cells by SA was not directly associated with the disease. This study lends support to the potential role of SA in the activation of MBP-reactive T cells and suggests that an altered regulatory mechanism may account for further expansion and persistence of MBP-reactive T cells in MS.

Journal of Biochemical and Biophysical Methods (14)


http://www.sciencedirect.com/science/article/B6T28-46HJMPJ-2/2/d8f639256a9e5983ff8fceed0137e308

Although real-time PCR is a rapid, quantitative method for the analysis of gene and RNA levels, the presence of inhibitors in samples is an obstacle to its successful use. We have found that genomic DNA isolated from mouse tail tips using a standard proteinase K digestion method caused marked inhibition of real-time PCR. Inhibition was specific for mouse tail DNA since genomic DNA isolated from other tissue sources using the same methodology was readily amplified. We have therefore developed a nonproteinase K DNA isolation method involving the use of Chelex(R) 100 resin. This method produces mouse tail DNA that is free of real-time PCR inhibitors.


http://www.sciencedirect.com/science/article/B6T28-47NM5J-6/2/cd2c380957833a438c4147fddac6673e

The preparation and purification of PCR generated DNA fragments suitable for footprinting and classical drug binding studies is described. One of the fragments, a 214-mer derived from pBR322 DNA exhibits a biphasic melting profile. This behavior appears to be due to a non-random distribution of base pairs within the fragment causing a region rich in AT base pairs to melt prior to a segment having a high concentration of GC base pairs. The usefulness of large amounts of PCR generated DNA for footprinting and optical binding studies involving drugs is
also presented and discussed.


http://www.sciencedirect.com/science/article/B6T28-429Y53Y-C/2/6fe14bea71f71b178da1b9f60cc4b7df

By testing DNA pools rather than single samples the number of tests for a case-control association study can be decreased to only two for each marker: one on the patient and one on the control pool. A fundamental requirement is that each pool represents the frequency of the markers in the corresponding population beyond the influence of experimental errors. Consequently the latter must be carefully determined. To this aim, we prepared pools of different size (49-402 individuals) with accurately quantified DNAs, estimated the allelic frequencies in the pools of two SNPs by primer extension genotyping followed by DHPLC analysis and compared them with the real frequencies determined in the single samples. Our data show that (1) the method is highly reproducible: the standard deviation of repeated determinations was +/-0.014; (2) the experimental error (i.e., the discrepancy between the estimated and real frequencies) was +/-0.013 (95% C.I.: 0.0098-0.0165). The magnitude of this error was not correlated to the pool size or to the type of SNP. The effect of the observed experimental error on the power of the association test was evaluated. We conclude that this method constitutes an efficient tool for high-throughput association screenings provided that the experimental error is low. We therefore recommend that before a pool is used for extensive association studies, its quality, i.e., the experimental error, is verified by determining the difference between estimated and real frequencies for at least one marker.


http://www.sciencedirect.com/science/article/B6T28-49JHR0K-4/2/cb03d8f49eb3270582a36a35c7fde837

Better understanding of the mechanisms involved in adipose tissue growth and metabolism is critical for the development of more effective treatments for obesity. However, because of its high lipid and low protein content, adipose tissue can present unique problems in some experimental procedures. We describe three protocols that provide new or improved methods for analysis of DNA, RNA, and protein from different adipose tissues. The first protocol provides a simple and rapid method for separation of fragmented DNA and visualization of apoptotic DNA laddering without the need for radioisotopes. This technique allows for an estimate of the amount of DNA fragmentation, and hence, apoptosis. The second protocol details subcellular fractionation of adipose tissue for the extraction of protein in the mitochondrial and cytosol fractions and the measurement of apoptotic protein (Bcl-2 and Bax) levels in each fraction. The last protocol involves extraction of total RNA from adipose tissue and the measurement of uncoupling protein mRNA using real-time RT-PCR, a method that has not previously been used to measure expression of uncoupling proteins in adipose tissue.


http://www.sciencedirect.com/science/article/B6T28-429Y53Y-2/2/8b095b042b00341d566dae95d00f5604

The high resolving power of the chromatographic separation of single- and double-stranded nucleic acids in 200 [μm] i.d. monolithic poly(styrene-divinylbenzene) capillary columns was utilized for mutation screening in polymerase chain reaction amplified polymorphic loci. Recognition of mutations is based on the separation of homo- and heteroduplex species by ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC) under partially denaturing conditions, resulting in characteristic peak patterns both for homozygous and heterozygous samples. Six different single nucleotide substitutions and combinations thereof were confidently identified in 413 bp amplicons from six heterozygous individuals each of which yielded a different unique chromatographic profile. Alternatively, mutations were identified in short, 62 bp PCR products upon their complete on-line denaturation at 75[deg]C taking advantage of the ability of IP-RP-HPLC to resolve single-stranded nucleic acids of identical length that differ in a single nucleotide. Separations in monolithic capillary columns can be readily hyphenated to electrospray ionization mass spectrometry and promise increased sample throughput by operating in arrays similar to those already used in capillary electrophoresis.


http://www.sciencedirect.com/science/article/B6T28-3Y2FV5D-8/2/ee6a7e7dcb66b95b444a76937936a61d

In the present investigation, oligonucleotide primers of high hybridisation stringency have been used in combination with optimised reverse transcriptase-polymerase chain reaction (RT-PCR) methods for the determination of the cDNA sequence corresponding to porcine FGF-2 mRNA present in brain and uterine tissue. Application of these optimised methods have overcome previous limitations associated with the low abundance of the porcine FGF-2 mRNA, and allowed as little as 100 [μg] of tissue to be employed to generate the complete cDNA nucleotide sequences as well as to provide specific template fragments selected for their suitability in subsequent ligation and mutagenesis studies with conventional expression vectors. Comparisons of the cDNA nucleotide and the deduced amino-acid sequence of porcine FGF-2 and the known FGF-2s from other species have indicated nucleotide sequence homologies of 95.5% with the bovine, 94.7% with the human and 88.7% with the rat FGF-2 cDNA whilst amino-acid sequence homologies of 100% with the bovine, 98.7% with the human and 96.8% with the rat FGF-2, respectively, were found. Based on these investigations, application of analogous strategies and methods with low abundance mRNAs related to other members of this family of growth factors, as well as very low abundance mRNAs of other protein growth factor, in the pig should now be readily realised.


http://www.sciencedirect.com/science/article/B6T28-429Y53Y-D/2/bdeb926e5ac4cb9aa646cd408b5ba262
We have investigated the possibility of determining quantitatively the alleles of binary DNA polymorphisms by single-nucleotide primer extension (SNuPE) and fluorescence-based DHPLC. Using a polymorphism of interest to our group, ROX-labeled dideoxy CTP (ROX-ddCTP) was incorporated at the 3' end of the primer annealed to the template adjacent to the polymorphic site. The primer extension product was then resolved from the unincorporated dye terminator by ion-pair reversed-phase liquid chromatography. The signal intensity of incorporated ROX-ddCTP correlated well over one order of magnitude with the relative amount of the C-allele present in the genomic DNA template. We conclude that SNuPE, when combined with fluorescence-based DHPLC, can accurately determine the relative molar proportion of one allele in total DNA.


A variety of methods for high throughput analysis of differential gene expression has been developed over the past years. We have implemented the EASEL technique that adds flexibility, efficiency and wide-applicability to these methods. The EASEL procedure is unique as it integrates several well established techniques and thereby offers a combination of subtractive hybridization of 3' cDNA ends with macroarrays analysis and Serial Analysis of Gene Expression (SAGE). In addition, once a set of interesting, differentially expressed genes is identified, the material required for follow up studies to test the hypothesis that the gene is truly involved in the process of interest is readily available. In this report, we first present a step-by-step validation of the procedure, since several of the incorporated steps had to be tailored to meet specific requirements and implied drastic modifications of the original methods. Secondly, we applied EASEL to the identification of up-regulated gene products in the outflow tract region of the embryonic rat heart. Here we provide evidence that at least two among the differentially expressed genes detected, follistatin-like protein gene and membrane type 1-metallo proteinase gene, are selectively up-regulated in the outflow tract, suggesting their involvement in the development of this region during embryogenesis.

extents by intercalating dyes and, in some PCR systems, intercalating dyes at unusually high concentrations maintain input DNA electrophoretic profile.


http://www.sciencedirect.com/science/article/B6T28-46HJMPJ-1/2/a26d61dd3fd4580c69894558dfd3f6a6

A new one-step method for fast and efficient preparation of double-stranded DNA template, suitable for use with Pyrosequencing(TM) technology, has been developed. In the new method, two different types of oligonucleotides were used to prevent reannealing of remaining PCR primers to the template: oligonucleotides complementary to the PCR primers and 3'-end modified oligonucleotides with the same sequence as the PCR primers. Advantages with the new strategy are: (i) faster and simpler template preparation procedure (one-step); (ii) no need for exonuclease I treatment; and (iii) less problem with unspecific priming from loop structures and dimers. By careful oligonucleotide design, and/or by addition of single-stranded DNA-binding protein, problems with unspecific sequence signals due to mispriming can be reduced. The new method was used for analysis of genotype variations within the renin-angiotensin-aldosterone system.


http://www.sciencedirect.com/science/article/B6T28-429Y53Y-4/2/94f0c3b5a800e14a5b834f4c502b0815

Sensitive and automated methods for the detection of DNA sequence variation are required for a wide variety of genetic studies. Diagnostic testing in human genetic disorders is one application of such methods. Tuberous sclerosis complex (TSC) is an autosomal dominant familial tumor syndrome characterized by the development of benign tumors (hamartomas) in multiple organs (OMIM # 19110, #191092). There is a high frequency of sporadic cases and significant demand from patients and families for genetic testing information. Two TSC genes have been identified (TSC1 and TSC2) and together account for all cases [1 and 2]. Here we report our methods for DHPLC analysis of the TSC1 gene and demonstrate the high sensitivity of this method in a blinded analysis of 21 TSC patients with known TSC1 mutations. In this series, DHPLC detected 27/28 (96%) known TSC1 sequence variations. The only sequence variation not identified by DHPLC in this study is a mosaic case.


http://www.sciencedirect.com/science/article/B6T28-47MSRBD-1/2/e903600f32d9314a752639b8ea02cad7

The small quantities of tissue available for most studies of human disease are a significant limitation for meaningful gene expression profiling. The Atlas Switch Mechanism At the 5' end of
Reverse Transcript (SMART(TM)) probe amplification kit uses as little as 50 ng of total RNA to generate complex cDNA probes for DNA array and other analyses. However, the extent to which this attractive methodology maintains representation of relative gene expression has not been quantified. In this study, we demonstrate using real-time quantitative PCR analysis that the relative expression levels of a range of low- to high-abundance mRNAs are retained after SMART amplification independent of transcript abundance and full-length transcript, coding region and PCR product size. Using this technology, a mean amplification of 3800-fold was achieved in human liver samples, greatly enhancing the ability to perform replicate DNA array experiments. Probes generated with the SMART amplification method were used to detect increased expression of genes involved with inflammation, fibrosis, xenobiotic metabolism, immune function, oxidant stress and endothelium in liver from the baboon model of alcoholic liver disease.


http://www.sciencedirect.com/science/article/B6T28-4F473MF-1/2/12e5c2652c39908f0fa0af90410868f0

A number of PCR-based in situ hybridization (ISH) techniques have been reported to facilitate the procedure. However, those techniques require additional gene specific primers with RNA polymerase binding site. We developed a new PCR-based ISH technique without extra gene-specific primers. We amplified gene specific PCR products with regular gene-specific primer pairs. Special linker, including T7 RNA polymerase binding site, was adapted to amplified PCR products. Secondary PCR was performed with T7 primer, and forward or reverse primer, used for the first PCR to prepare template DNA for RNA transcription. We were able to generate sense and anti-sense probes for ISH in a day. Recently, real-time PCR and ISH are required to validate microarray results quantitatively and qualitatively. This technique can be expected to facilitate the high-throughput validation of transcripts detected by microarrays.


http://www.sciencedirect.com/science/article/B6T28-3RC4TD2-1/2/bb6e63c5486309c3e3e295f45a1eb451

We describe an approach, which combines the process of DNA amplification and sequence determination by using a pair of primers and two DNA polymerases with different incorporation rates for dideoxynucleotides. The process of target sequence amplification is carried out by the DNA polymerase with a low dideoxynucleotide incorporation rate while its polymerase counterpart with a high incorporation rate generates a sequence ladder. The needs for separate amplification via polymerase chain reaction (PCR) or cloning into plasmids including the respective purification steps therefore can be avoided. In addition, the use of dye terminator chemistry enables the simultaneous generation of forward and reverse sequence ladders, which can be separated based on the streptavidin-biotin system when one amplification primer is biotinylated.

Journal of Biomechanics (1)
Bone is subjected in vivo to both high amplitude, low frequency strain, incurred by locomotion, and to low amplitude, broad frequency strain. The biological effects of low amplitude, broad frequency strain are poorly understood. To evaluate the effects of low amplitude strains ranging in frequency from 0 to 50 Hz on osteoblastic function, we seeded MC3T3-E1 cells into collagen gels and applied the following loading protocols for 3 min per day for either 3 or 7 days: (1) sinusoidal strain at 3 Hz, with 0-3000 [mu]strain peak-to-peak followed by 0.33 s resting time, (2) "broad frequency vibration" of low amplitude strain (standard deviation of 300 [mu]strain) including frequency components from 0 to 50 Hz, and (3) sinusoidal strain combined with broad frequency vibration (S+V). The cells were harvested on day 4 or 8. We found that the S+V stimulation significantly repressed cell proliferation by day 8. Osteocalcin mRNA was up-regulated 2.6-fold after 7 days of S+V stimulation, and MMP-9 mRNA was elevated 1.3-fold after 3 days of vibration alone. Sinusoidal stimulation alone did not affect the cell responses. No differences due to loading were observed in alkaline phosphatase activity and in mRNA levels of type I collagen, osteopontin, connexin 43, MMPs-1A, -3, -13. These results suggest that osteoblasts are more sensitive to low amplitude, broad frequency strain, and this kind of strain could sensitize osteoblasts to high amplitude, low frequency strain. This suggestion implies a potential contribution of stochastic resonance to the mechanical sensitivity of osteoblasts.


Lysobacter sp. IB-9374, which was isolated from soil as a high lysyl endopeptidase-producing strain (Chohnan et al., FEMS Microbiol. Lett., 213, 13-20, 2002), was found to produce a [beta]-lytic protease capable of lysing gram-positive bacteria such as Staphylococcus aureus, Micrococcus eus, and Bacillus subtilis. The Lysobacter strain secreted the [beta]-lytic protease into the culture medium at a 2.4-fold higher level than Achromobacter lyticus. The enzyme was highly purified through a series of six steps with a high yield. The enzyme was strongly inhibited by tetraethylene-pentamine and 1,10-phenanthroline. The purified enzyme lysed more efficiently almost all the gram-positive bacteria tested than lysozyme, lysostaphin, and mutanolysin. The enzyme was very similar to Achromobacter [beta]-lytic protease containing one zinc atom in terms of amino acid composition and N-terminal sequence. The nucleotide sequence revealed that the mature enzyme was composed of 179 amino acid residues with additional 198 amino acids at the amino-terminal end of the enzyme. The deduced amino acid sequence of the mature enzyme coincided with that of the Achromobacter enzyme, although the prepro-region showed a 41% sequence identity with the counterpart. These results indicate that Lysobacter sp. is a useful strain for an efficient large-scale preparation of [beta]-lytic protease capable of lysing bacteria.


The enrichment and characterization of anaerobic ammonium-oxidizing biofilm cultures are ongoing in our laboratories. Biomass, with a predominately red color, demonstrating simultaneous removal of ammonium and nitrite under autotrophic and anoxic conditions, which is characteristic of anaerobic ammonium-oxidizing planctomycetes, was enriched and maintained for an extended period on a polyester nonwoven carrier. To investigate the bacterial composition of the mature biofilm community, 16S rDNA sequences were amplified by PCR and comparative analyses using DNA databases were conducted. Only one sequence had a notable similarity (92.2%) to that of the first discovered anaerobic ammonium-oxidizing planctomycete and lesser, yet significant, similarities to the 16S rDNA sequences of other recently reported anaerobic ammonium-oxidizing strains. The newly discovered strain (designated KSU-1) reported here was dominant among detectable members of the biofilm community. By fluorescence imaging, KSU-1 was shown to form spherical clusters wrapped in a thin layer of Zoogloea sp. Possible interactions and interdependencies of these two species are discussed with regard to the putative unculturability of the anaerobic ammonium-oxidizing planctomycetes.


http://www.sciencedirect.com/science/article/B6VSD-43CTKX4-D/2/929454cd3e5a3820325c2ebbae3ed357

Two extremely thermophilic alkane-degrading bacterial strains, B23 and H41, were respectively isolated from deep subterranean petroleum reservoirs in the Minami-aga (Niigata) and Yabase (Akita) oil fields. Both strains were able to grow at temperatures ranging from 50 to 80[deg]C, with optimal growth at 70[deg]C for B23 and 65[deg]C for H41. From 16S rRNA gene sequence analysis and physiological characterization, both strains were identified as Bacillus thermoleovorans (identities of 99.5% and 99.6% to strain DSM 5366, and 98.3% and 98.7% to the type strain LEH-1TS, respectively). Strains B23 and H41 effectively (60%) degraded n-alkanes longer than C12 and C15, respectively, at 70[deg]C, while strain LEH-1TS degraded undecane (C11) most effectively. When B23 and H41 were cultivated in the presence of heptadecane, heptadecanoate and pentadecanoate were specifically accumulated in the cells. These results strongly suggest that the two strains degraded n-alkanes by a terminal oxidation pathway, followed by a [beta]-oxidation pathway.


http://www.sciencedirect.com/science/article/B6VSD-4FB66DB-B/2/6c84c70a46d677c72124dd0522a0aa39
An aryl phosphate ester (APE)-degrading bacterium was isolated from the leachate of a sea-based waste disposal site. The isolated APE-degrading bacterial strain YS-57 grew well in a medium containing glucose and NaCl, and degraded two types of APE: tricresyl phosphate and triphenyl phosphate. The optimal temperature, pH, and NaCl concentration for the growth of strain YS-57 were 30[°C], 7.0, and 1.0%, respectively. Strain YS-57 grew at an APE concentration of 25 mg/l without being inhibited. APEs were degraded by the supernatant of the medium in which strain YS-57 was cultured, suggesting that the APE-degrading enzyme was released into the extracellular space in the logarithmic growth phase. The 16S rDNA sequence of strain YS-57 showed 95.6% similarity to that of Roseobacter gallaeciensis and the morphological properties were also comparable. Consequently, strain YS-57 was closely related to the genus Roseobacter.


Di-fructofuranose-1,2',2,3'-dianhydride (DFA III) was shown to enhance Ca absorption in rat and human intestine. The effects of DFA III administration (9 g per day for 4 weeks that corresponded to 3-fold the optimal dosage of DFA III) on human intestinal microbiota were studied using denaturing gradient gel electrophoresis (DGGE). The major groups of human intestinal microbiota reported previously: the Bacteroides, the Clostridium cocoides group (Clostridium cluster XIVa), the Clostridium leptum group (Clostridium cluster IV), and the Bifidobacterium group were detected. The similarity of 30 DGGE profiles based on the V3 region (before and after administration to the 15 subjects) of the 16S rDNA were calculated using Pearson's correlation based on numbers, positions and intensity of bands, and then a dendrogram of DGGE profiles was constructed by the unweighted pair group method using arithmetic average (UPGMA) clustering method. By these analyses, no difference in DGGE profiles after DFA III administration was observed in healthy subjects, while two subjects with chronic constipation showed different profiles, namely on numbers, positions and the intensity of some bands. Their stools were softer and stool frequencies increased and they obtained relief from constipation.

http://www.sciencedirect.com/science/article/B6VSD-41XVBHW-6/2/1ac265263170e5445202f4e0e2271b65

The sensitivity of a methanogen and sulfate-reducing bacterium isolated from a sea-based landfill site to Cd2+ and Cu2+ was studied. Methanogens and sulfate-reducing bacteria in leachates of the waste disposal site were enumerated using the MPN method. Methanobacterium thermoautotrophicum KHT-2, isolated from the leachate, could not grow at 0.5 mM Cd2+ or 1.0 mM Cu2+. Desulfotomaculum sp. RHT-3, isolated from the same leachate, was able to insolubilize 3.0 mM Cd2+ or 2.0 mM Cu2+ by production of hydrogen sulfide. When strains KHT-2 and RHT-3 were cultured together in the presence of the heavy metals, strain KHT-2 could grow at high heavy metal concentrations after insolubilization of the metals by strain RHT-3.


http://www.sciencedirect.com/science/article/B6VSD-426YS50-N/2/b7e99e44285b101e467aded8c40d3e66

We have previously reported that a protein library can be constructed by directly combining PCR amplification of a single DNA molecule and cell-free protein synthesis. To specifically amplify single DNA molecules, however, two-step PCR with nested primers was used. Here we describe a simpler method for single-step amplification of a single molecule. The method involves the use of both hot-startable DNA polymerase and a DNA template that has homo-priming sequences at both ends for amplification using a single primer. These two modifications greatly decreased the possibility of formation and subsequent accumulation, respectively, of primer-dimers that inhibit the amplification of target template. In addition, a high-fidelity DNA polymerase was successfully used, resulting in the significant reduction of the accumulation of mutations during amplification.


http://www.sciencedirect.com/science/article/B6VSD-49D202X-8/2/1bb08b5d0ffa9cdb9e4235d382c0a7bb

A self-heating field-scale composter treating agro-industrial wastes within a period of 30 d was analyzed by denaturing gradient gel electrophoresis (DGGE) (Pedro et al., J. Biosci. Bioeng., 91, 159-165, 2001). Three major bands were derived from Propionibacterium acnes, Methylobacterium mesophilicum or M. radiotolerans, and Bacillus thermocloacae. Strains MSP09A and MSP06G with close affiliation to P. acnes and B. thermocloacae, respectively, were successfully isolated. Based on quantitative-PCR results, the relative population of MSP09A increased towards the end of the composting process (mesophilic stage) while MSP06G seemed to predominate during the middle period (thermophilic stage). These results correlated highly with their growth temperatures. MSP09A and MSP06G had different metabolic profiles which were largely affected by culture conditions. MSP09A was able to utilize large complex molecules of lipids and proteins. An interspecies relationship in terms of metabolites such as propionic acid
was expected between the two microorganisms.


http://www.sciencedirect.com/science/article/B6VSD-431BBWD-9/2/25a8761c69c583fa0c0e8e515c6e065a

The diversity of microbial community during the decomposition of waste in a field-scale composter (Hazaka system) was investigated by denaturing gradient gel electrophoresis (DGGE). The composter operates at a high temperature through a self-heating system, creating a thermophilic (60-76°C) stage during the initial phase and a mesophilic (45°C) stage towards the later phase of the composting period. The pH of the system (pH 7.75-8.10) did not vary significantly during the process while moisture content was reduced from 48.8% to 25.1%. DGGE and 16S rDNA analyses showed that the following genera were found throughout the process: Propionibacterium sp., Methylobacterium sp., Pseudomonas sp., and Bradyrhizobium sp. Different Bacillus spp. thrive at the thermophilic or the mesophilic stage while Clostridium sp. was only found at the initial phase of the process. Staphylococcus sp. and Caulobacter sp. or Brevundimonas sp. existed during the later phase of the composting period.


http://www.sciencedirect.com/science/article/B6VSD-4BCWP93-5/2/baa6010b89632bd885fa91aadb7a62f5

Recently, the use of the dry yeast of Zygosaccharomyces rouxii M2 for miso (soybean paste) fermentation has been established. A molecular monitoring method was developed and validated in this study to analyze the population of Z. rouxii M2 during the fermentation. The method was based on the restriction patterns of internal transcribed spacer (ITS) regions of the rDNA using HaellI and HhaI. Among the homologous ITS regions of Z. rouxii strains, Z. rouxii M2 produced diagnostic bands by which it can be differentiated from the other strains used. The specific restriction bands were due to the difference in nucleotide sequence of two different copies of ITS of Z. rouxii M2. Both ITS copies showed 94% sequence similarity but a 13-bp nucleotide substitution and a 19-bp deletion were found in the ITS1 region. Phylogenetic trees were constructed based on ITS and 18S rDNA sequences and it was found that the ITS sequences provide better resolution for the classification of Z. rouxii M2. Since Z. rouxii M2 is a promising strain for use in miso fermentation as a dry starter, the method developed is significant in terms of industrial application in monitoring the growth of Z. rouxii M2 in miso fermentation.


http://www.sciencedirect.com/science/article/B6VSD-4FPN41CC/2/bdba62d68269b852bb0901795855e6a1
The gut contents and faeces of 9 adult pigs and 387 colonies growing on MRS culture (selective for Lactobacillus) were sampled to isolate and identify Lactobacillus species present. The results showed that the number of bacteria growing on MRS culture increased from the anterior to posterior of the gut and decreased in the faeces (e.g., stomach, 4.04 x 10^5 cfu/g; small intestine, 9.77 x 10^7 cfu/g; large intestine 3.85 x 10^11 cfu/g; faeces, 1.22 x 10^11 cfu/g). Fifty-two lactobacilli-like colonies were selected from 387 on the basis of their size and Gram staining, and used for the fermentation of 11 carbohydrates, from which 12 lactobacilli were selected for 16S rDNA analysis. The result showed that Lactobacillus ruminis was the dominant Lactobacillus in the stomach, small intestine, large intestine and faeces of pigs. The identification of most strains estimated by their 16S rDNA was 98-99% accurate.


http://www.sciencedirect.com/science/article/B6VSD-49GYVN6-1R/2/c472f5041ed10e830ce1c1e1c805ddbf

Bacterial and artificial magnetic particles were modified using a polyamidoamine (PAMAM) dendrimer and outer shell amines determined. Bacterial magnetic particles were the most consistently modified. Transmission electron microscopic (TEM) analysis showed that the artificial magnetic particles were structurally damaged by the modification process including sonication. Furthermore, laser particle analysis of the magnetite also revealed damage. Small quantities of dendrimer-modified bacterial magnetic particles were used to extract DNA from blood. The efficiency of DNA recovery was consistently about 30 ng of DNA using 2-10 [mu]g of dendrimer-modified bacterial magnetite. This technique was fully automated using newly developed liquid handling robots and bacterial magnetic particles.

Journal of Biotechnology (18)


http://www.sciencedirect.com/science/article/B6T3C-4BSVBH3-1/2/c4e9bc3a418367b798304506578fbdff

White spot syndrome, caused by white spot syndrome virus (WSSV), is a deadly disease of shrimps, causing a catastrophic loss in shrimp industries worldwide. In order to investigate molecular response of shrimp haemocyte to WSSV infection, we performed subtraction hybridization of mRNAs from healthy and WSSV-infected haemocyte. One of the genes that were severely down-regulated in moribund WSSV-infected-haemocyte was translationally controlled tumor protein (TCTP) (or fortillin). Strikingly, while there was a slight difference in the amount of TCTP message between normal and early WSSV-infected shrimps, shrimps that exhibited severe symptoms uniformly had very little TCTP in their haemocyte. Taken together with the fact that TCTP functions as an anti-apoptotic protein in mammals, our data suggest that TCTP in shrimp protects WSSV-infected shrimps from death.

http://www.sciencedirect.com/science/article/B6T3C-4DNB0SC-1/2/9b91c5ad283767c1ef860a53ee0ff38b

Electronic microarray technology is a potential alternative in bacterial detection and identification. However, conditions for bacterial detection by electronic microarray need optimization. Using the NanoChip electronic microarray, we investigated eight marine bacterial species. Based on the 16S rDNA sequences of these species, we constructed primers, reporter probes, and species-specific capture probes. We carried out two separate analyses for longer (533 bp) and shorter (350 and 200 bp) amplified products (amplicons). To detect simultaneously the hybridization signals for the 350- and 200-bp amplicons, we designed a common reporter probe from an overlapping sequence within both fragments. We developed methods to optimize detection of hybridization signals for processing the DNA chips. A matrix analysis was performed for different bacterial species and complementary capture probes on electronic microarrays. Results showed that, when using the longer amplicon, not all bacterial targets hybridized with the complementary capture probes, which was characterized by the presence of false-positive signals. However, with the shorter amplicons, all bacterial species were correctly and completely detected using the constructed complementary capture probes.


http://www.sciencedirect.com/science/article/B6T3C-42D2C33-2/2/10035fdf5e07870efb6874c8619c7ce4

Pseudomonas alcaligenes secretes a lipase with a high pH optimum, which has interesting properties for application in detergents. The expression of the lipase is strongly dependent on the presence of lipids in the growth medium such as soybean oil. The promoter of the gene was characterized and found to have resemblance to [sigma]54 controlled promoters, which are known to be tightly regulated. The transcription start was mapped precisely downstream of a sequence with close similarity to the -12/-24 consensus sequence of [sigma]54 controlled promoters. Interestingly, a hyperproducer mutant strain was isolated and found to have a C to T mutation in the -12/-24 promoter consensus region. In addition an Upstream Activating Sequence (UAS) with homology to [sigma]54 UAS consensus sequences was identified. It was demonstrated that an increase of the distance from the UAS to the transcription start or the deletion of the UAS results in significantly lower expression levels of lipase. A systematic mutational analysis of the UAS sequence has resulted in a variant with an increased lipase expression.


http://www.sciencedirect.com/science/article/B6T3C-3TXT1RT-4/2/087c52ad9fe7ed6bb35a2dc49dcad9a9

Pseudomonas alcaligenes M-1 has been selected from an intensive screening for micro-
organisms that can naturally produce a lipase active in detergent formulations. The lipase expression has been increased to allow high level secretion from Pseudomonas alcaligenes, via the introduction of multi-copy plasmids. In order to improve the lipase yield further, the phenotype enhancement method has been developed. This idea comprises the reintroduction of a cosmid library with random chromosomal fragments in a P. alcaligenes strain with already high lipase productivity. One of the strains which showed an enhanced lipase production appeared to contain a cosmid encoding the outer membrane secretion genes. These xcp-genes are clustered in two divergently transcribed operons similar to the situation in Pseudomonas aeruginosa. Remarkably and dissimilar to P. aeruginosa, in between the two xcp gene clusters, two reading frames of unknown function--OrfV and OrfX--are present. For OrfX no equivalent can be found in the known protein data bases. On the other hand, OrfV shows homology to the regulatory proteins MalT and AcoK. Some evidence is provided that suggests that OrfV acts as a regulator of the xcp operons. A model is proposed for the regulation of the secretion system from P. alcaligenes.


http://www.sciencedirect.com/science/article/B6T3C-497RD6K-5/2/ac6be9a7b587218391573b694fc36425

In this study, ammonia-oxidizing bacteria present in biofilms resulting from a nitrifying reactor were detected by both a conventional FISH technique and an original in situ PCR technique. Both techniques showed that ammonia-oxidizing bacteria were found near the surface of the biofilms. However, after the biofilm had been exposed to 2 weeks of ammonia starvation, ammonia-oxidizing bacteria present in the biofilm could not be detected by fluorescence in situ hybridization (FISH) because they did not have sufficient copies of rRNA. In contrast, ammonia-oxidizing bacteria could be detected by in situ PCR with strong signal. It was thus demonstrated that a cell possessing a specific functional gene is detectable by in situ PCR regardless of its activity.


http://www.sciencedirect.com/science/article/B6T3C-47P1TYB-3/2/037704017bc58504efc5473a0da9615d

We have cloned and constructed plasmid vectors, pETB23H and pETB23L, for bacterial expression of heavy (H) and light (L) chain cDNAs of Fab' of mAbB23 a monoclonal antibody specific to human plasma apolipoprotein (apo) B-100. The H- and L-chains were expressed as insoluble inclusion bodies in the cytoplasm of Escherichia coli. The inclusion bodies of both chains were isolated from the cell lysate, solubilized in 6 M guanidium-HCl, and mixed in equal molar amounts. Refolding was performed in three stages of dialysis: first, dialysis against 3 M guanidium buffer, next, continuous decrement of guanidium in the dialysis buffer through slow addition of 1 M guanidium buffer, and finally, dialysis against a buffer without guanidium. After the refolding, active Fab' (rFab') was purified through an apo B-100-co coupled affinity column. When compared by ELISA, the rFab' had a slightly decreased antigen-binding activity (about 0.7-fold) compared with native Fab. The refolding yield was maximum (75%) when performed at the protein concentrations not more than 0.4 mg ml-1, whereas the yield decreased exponentially at higher concentrations. The maximum recovery was obtained at the refolding concentration of 1.8 mg ml-1, where the yield was about 45%. Overall, 2.4-3.0 mg of active rFab' specific to apo B-100 was successfully obtained from 1 l cultivation of E. coli cells.
Luciferase gene was introduced into fertilized eggs of medaka by a localized electric field between thin film electrodes formed on a glass plate. Miniaturization of the electrodes enabled us to apply a localized electric field to the animal pole of the fertilized egg. Biochemical luminescence and an electrophoresis pattern showed expression and integration of the gene, respectively. The presented system had a higher ratio of gene introduction than the conventional electroporation method.

A simple microchip device for DNA extraction was constructed based on electrostatic interactions between surface amine groups and DNA. Microchannel was fabricated on silicon wafer by photolithography and coated with 3-aminopropyltriethoxysilane (APTES) or 3-[2-(2-aminoethylamino)-ethylamino]-propyltrimethoxysilane (AEEA) to introduce amine groups on the surface. Determination of the number of surface amine groups and optimization of DNA capture condition were demonstrated to characterize the microchip. Capacities of capturing DNA were approximately 97 ng/cm² in APTES and 194 ng/cm² in AEEA modified microchips, respectively. The amount of DNA captured in the microchip increased depending on surface amine density. Furthermore, DNA extraction using amine-coated microchip from whole blood was examined. Quantification of DNA and proteins in washing or eluting fraction indicates that proteins were removed at washing steps and only DNA was effectively eluted by changing alkalinity of buffer from pH 7.5 to 10.6. The amount of DNA extracted from whole blood was approximately 10 ng and its recovery ratio was 27-40%. Performance of PCR for the eluted fraction indicates that DNA extracted from whole blood was well purified using amine-coated microchip.

The optimisation of enzymes for particular purposes or conditions remains an important target in virtually all protein engineering endeavours. Here, we present a successful strategy for altering the pH-optimum of the triglyceride lipase cutinase from Fusarium solani pisi. The computed electrostatic pH-dependent potentials in the active site environment are correlated with the experimentally observed enzymatic activities. At pH-optimum a distinct negative potential is present in all the lipases and esterases that we studied so far. This has prompted us to propose the 'The Electrostatic Catapult Model' as a model for product release after cleavage of the ester bond. The origin of the negative potential is associated with the titration status of specific residues.
in the vicinity of the active site cleft. In the case of cutinase, the role of Glu44 was systematically investigated by mutations into Ala and Lys. Also, the neighbouring Thr45 was mutated into Proline, with the aim of shifting the spatial location of Glu44. All the charge mutants displayed altered titration behaviour of active site electrostatic potentials. Typically, the substitution of the residue Glu44 pushes the onset of the active site negative potential towards more alkaline conditions. We, therefore, predicted more alkaline pH optima, and this was indeed the experimentally observed. Finally, it was found that the pH-dependent computed Coulombic energy displayed a strong correlation with the observed melting temperatures of native cutinase.

http://www.sciencedirect.com/science/article/B6T3C-4F0181B-1/2/9a0d5bb3b3b1e37686628b1fac5932af

We have developed a ready-to-spot polymer microarray slide, which is coated with a uniform layer of reactive electrophilic groups using anthraquinone-mediated photo-coupling chemistry. The slide coating reduces the hydrophobicity of the native polymer significantly, thereby enabling robust and efficient one-step coupling of spotted 5' amino-linked oligonucleotides onto the polymer slide. The utility of the coated polymer slide in gene expression profiling was assessed by fabrication of spotted oligonucleotide microarrays using a collection of 5' amino-linked 70-mer oligonucleotide probes representing 96 yeast genes from Operon. Two-colour hybridizations with labelled cDNA target pools derived from standard grown and heat-shocked wild type yeast cells could reproducibly measure heat shock induced expression of seven different heat shock protein (HSP) genes. Moreover, the observed fold changes were comparable to those reported previously using spotted cDNA arrays and high-density 25-mer oligonucleotide arrays from Affymetrix. The low hybridization signals obtained from the [Delta]SSA4 mutant cDNA target, together with the high signal detected in two-colour hybridizations with heat-shocked wild type yeast relative to the [Delta]SSA4 mutant strain implies that unspecific binding of cDNA target to the SSA4-specific 70-mer oligonucleotide probes is negligible. Combined, our results indicate that the coated polymer microarray slide represents a robust and cost-effective array platform for pre-spotted oligonucleotide arrays.

http://www.sciencedirect.com/science/article/B6T3C-4CC7WVX-6/2/267b1f79a8e1db483295394a168aceb3

The PCR-based genome walking method has been commonly used to isolate upstream regions from known cDNA sequences. The limitation of this technique is based on the location of the restriction site upstream to the gene-specific primer in the genome; hence, different restriction enzymes have to be used to isolate larger upstream fragments. In this paper, we present the advantageous use of partial and size-selected DNA as templates for genome walking, in isolating larger upstream fragments. We have successfully tested this approach to isolate larger upstream fragments using the FailSafe(TM) PCR System. Use of partial digestion and size selection can provide better chances in obtaining larger flanking regions of known DNA sequence, when compared to use of total digested DNA.

http://www.sciencedirect.com/science/article/B6T3C-3YTJKWB-C/2/2d633a9f26de1a00f8f9fc85542a92a3

The need to identify disease-causing mutations and DNA polymorphisms has increased with the continuing identification of new candidate genes. PCR single-strand conformation polymorphism (PCR-SSCP) is one of the techniques most widely used to identify a mutant sequence or a polymorphism in a known gene. However, the original SSCP protocols using the incorporation of radioactive label and polyacrylamide gel electrophoresis on sequencing gels for detection were labour intensive and time-consuming. Here we describe a simple SSCP protocol using MDE(TM) gel solution and a midi gel format to detect SSCP variations in the glucose transporter gene GLUT1, that we have previously analysed with the standard radioactive SSCP protocol, and we have also tested this method on the previously described point mutation (A/G transition in exon 1) of the CTLA-4 (cytotoxic T lymphocyte associated-4) gene. All known variants were detected. Based on the results, this technique appears to be simple, with no use of radioactive labels and with easy handling of the gel. Furthermore, it needs little optimisation, is relatively rapid and highly sensitive. We propose this method for the first screening for candidate gene variants.


http://www.sciencedirect.com/science/article/B6T3C-3WNMH42-B/2/bda75e276cf2918f6ce72faa372ba56e

The gene encoding extracellular phospholipase A1 of Serratia sp. MK1 was cloned from a genomic DNA library. Formation of transparent halos on the PCY agar plates was used to identify E. coli carrying the phospholipase A1 gene. A 4.2 kb EcoRI fragment was isolated and sequenced. From nucleotide sequences and expression of various plasmids, two open reading frames (plaA and plaS) involved in efficient expression of phospholipase A1 in natural and recombinant host were identified. Extracellular phospholipase A1 activity was identified as the gene product of plaA encoding 321 amino acids with a predicted MW of 33400. Analysis of the amino acid sequence revealed significant homology (around 70%) to phospholipase A1 of Serratia liquefaciens and Yersinia enterocolitica. The sequence, -Gly-X1-Ser-X2-Gly-, known as a lipase-specific consensus sequence was also found in the bacterial phospholipase A1. PlaS encoding a protein of 224 amino acids showed no enzymatic activity, but might be necessary for the efficient expression of phospholipase A1 in E. coli. To further improve the production of phospholipase A1 as a soluble and active form in E. coli, the effect of some parameters was examined. Surprisingly, a higher yield of soluble and active phospholipase A1 could be obtained under the combined conditions of a lower temperature, an enriched medium, and a lower-strength promoter.


http://www.sciencedirect.com/science/article/B6T3C-49P498R-1/2/7260c2aa61ea59939996c6e0941db0e

To improve detection efficiency and result accuracy, four screening primer pairs, four identifying
primer pairs, one common primer pair and corresponding probes were designed for the
development of multiplex polymerase chain reaction/membrane hybridization assay (MPCR-
MHA) for detection of the foreign genes insert in genetically modified organisms (GMOs). After
detecting condition and parameter were optimized and determined, MPCR reactions were
developed for amplifying several target genes simultaneously in one tube. Primers were labeled
with biotin at the 5'-end; biotinylated MPCR products were detected by hybridization to the
oligonucleotide probes immobilized on a membrane with subsequent colorimetric detection to
confirm hybridization. The testing of screening primers can judge whether the sample contains
GMOs, and that of identifying primers can further judge what kinds of trait genes are contained in
the sample. We detected nine soybean samples, six maize samples, seven potato samples and
two rice samples by the MPCR-MHA method; at the same time we also detected them with single
PCR-MHA method. The results between two methods have good consistency.

oxidoreductase (catA) gene from Brevibacillus choshinensis: stimulation of human epidermal

http://www.sciencedirect.com/science/article/B6T3C-48GVH3-6/2/cf8c06067d9f1b5e7d6e746e4eb84f1

Brevibacillus choshinensis (Bacillus brevis) is a protein-hyperproducing bacterium with a useful
host-vector system for the production of recombinant proteins. Here, we cloned the ccdA-catA ("|
height="11" width="9">cdA associated thioredoxin-like thiol-disulfide oxidoreductase) locus of B.
choshinensis HPD31-S5. CatA protein (molecular weight, 19664) contains a thioredoxin-like
motif, Cys-Gly-Pro-Cys. It was successfully expressed in B. choshinensis extracellularly (~100
[μg] ml-1 culture) using the secretion vector pNCMO2, and in Escherichia coli intracellularly
(~350 [μg] ml-1 culture) with an amino-terminal His-tag. Both recombinant proteins showed
thiol-disulfide oxidoreductase activity. Incubation of non-native human epidermal growth factor
(hEGF) containing incorrect disulfide bonds with B. choshinensis cells secreting CatA protein
resulted in the stimulation of the conversion of non-native hEGF to the native form. Furthermore,
co-expression of CatA protein with recombinant hEGF in the B. choshinensis production system
increased the yield of native hEGF.

contractor gene expression levels by a real-time PCR system." Journal of Biotechnology 84(2):
187.

http://www.sciencedirect.com/science/article/B6T3C-41PNYNT-B/2/f46de637f24478cee1413c577f222161

A rapid quantitative analysis method for murine endothelin-1 (ET-1) and vasoactive intestinal
contractor (VIC) gene expression levels was established using a real-time polymerase chain
reaction (PCR). We designed primer pairs and TaqMan probes specific for murine prepro-ET-1
(PPET-1) and prepro-VIC (PPVIC) genes, based on the cDNA sequence region common to both
mouse and rat. The dynamic range for detection in this system spanned 100000-fold of the
starting molecule. The gene expression levels of PPET-1 and PPVIC were estimated as gene
expression rates normalized by the expression of the house-keeping gene, glyceraldehyde-3-
phosphate dehydrogenase. To examine the reproducibility of this assay system, we calculated
the intra-assay and interassay coefficients of variation of the gene expression rate, which ranged
from 16.2 to 55.0% and from 24.2 to 56.5%, respectively. Using this system, we examined gene
expression levels of PPET-1 and PPVIC in mouse tissues. PPET-1 gene expression was found in
all tissues at relatively high levels, whereas high levels of PPVIC gene expression were observed
only in stomach, intestine, uterus, and ovary. The gene expression patterns agreed well with those determined by RNase protection assay and conventional PCR. These results show that this new rapid method is accurate and reproducible.


http://www.sciencedirect.com/science/article/B6T3C-47XSY7N-1/2/e186b5386c9d84fc14fc9fc7c5d8c98d

GeneTag(TM) is a novel expression profiling method that allows the visualization, quantification and identification of expressed genes--whether known or novel--in any species, tissue or cell type, independent of knowledge of the underlying sequence. Here we describe the application of this method to determine variation of gene expression in individual human liver samples and the identification of tissue-specific genes by comparing expression patterns across several human organs. Expression data are stored in a database for future reference and data analysis relies on proprietary software, which allows complex comparisons to be performed. Differentially expressed genes are quickly identified through a link to a sequence database. The results from our study underscore the importance of knowledge of individual variation of gene expression for the design and interpretation of transcript profiling experiments in the context of any biological question.


http://www.sciencedirect.com/science/article/B6T3C-47XFYR8-1/2/1f7a9b43f20e768ea87edf2a7d008265

A cascading hyperbranched polyamidoamine dendrimer was synthesized on the surface of bacterial magnetite from Magnetospirillum magneticum AMB-1 to allow enhanced extraction of DNA from fluid suspensions. Characterization of the synthesis revealed linear doubling of the surface amine charge from generations one through five starting with an amino silane initiator. Furthermore, transmission electron microscopy revealed clear dispersion of the single domain magnetite in aqueous solution. The dendrimer modified magnetic particles have been used to carry out magnetic separation of DNA. Binding and release efficiencies increased with the number of generations and those of bacterial magnetite modified with six generation dendrimer were 7 and 11 times respectively as many as those of bacterial magnetite modified with only amino silane.

Journal of Cereal Science  (3)

Timing of transcript accumulation for genes involved in a variety of cellular processes was assessed by RT-PCR in endosperm from developing wheat grains grown under moderate (24/17 [deg]C day/night) and high (37/28 [deg]C day/night) temperature regimens. Under moderate temperatures, transcripts for proteins with storage functions were present at all five time points examined between 7 and 34 DPA, while transcripts for proteins involved in signal transduction, protein synthesis and metabolism were most abundant from 7 to 20 DPA. Transcripts for proteins that play roles in defense were present from 14 DPA, about the time that starch accumulation commenced, to 34 DPA. High temperatures advanced and compressed the timing of transcript accumulation during grain development. Comparisons of transcript profiles with the timing of key events in grain development identified genes whose transcripts were accumulated at equivalent stages under the two temperature regimens and might serve as markers for grain development. These comparisons also revealed a number of genes with transcript profiles that were shifted under high temperatures in a manner that was not consistent with developmental events. These genes may be involved in responses to high temperature that are distinct from effects on the timing of developmental processes.


Puroindoline genotypes (Pina and Pinb) and their encoded proteins related to grain hardness were studied in various common wheat cultivars from Australia, China, Japan, Korea and North America. Most of the hard wheats had the Pinb-D1b genotype with a glycine to serine mutation at position 46. In addition to the known Pina and Pinb genotypes, cultivars were found with Pina and Pinb double-null mutations (Pina-D1b/Pinb-D1h (t)) and a new Pinb frameshift mutation (designated Pinb-D1i (t)) within the region encoding a tryptophan-rich domain. This new Pinb frameshift mutation was found only in Chinese cultivars. Endosperm proteins encoded by Pina and Pinb in these cultivars were analysed by 2D-gel electrophoresis (IPGXSDS-PAGE). Cultivars with Pina and Pinb double-null mutations showed no PIN-a or PIN-b protein, and cultivars with Pinb-D1i (t) had no PIN-b protein. Surprisingly, cultivars with Pinb-D1b had severely reduced amounts of PIN-b and cultivars with Pinb-D1c showed no PIN-b proteins. Grain hardness among cultivars having mutated Pinb may be explained by the amount of PIN-b protein and not by the type of amino acid substitutions.


A near-isogenic line (NIL) into which low-molecular-weight glutenin subunits (LMW-GSs) were introduced from a Canadian Western Extra-Strong wheat cultivar (Triticum aestivum L.), 'Glenlea', into a Japanese spring wheat cultivar, 'Harunoakebono', had a much better bread-making quality than does Harunoakebono. LMW-GSs associated with good bread-making quality of the NIL and the allelic LMW-GS in Harunoakebono were monitored by two-dimensional polyacrylamide gel electrophoresis in BC5F2 progenies derived from a cross between Harunoakebono and Glenlea.
The results show that LMW-GSs associated with good bread-making quality consist of five LMW glutenin components that co-segregate in a segregating population and that the allelic LMW-GS derived from Harunoakebono is also composed of five LMW glutenin components that co-segregate. N-terminal amino acid sequences of the five LMW glutenin components start with serine residues (LMW-s). Reverse transcription-polymerase chains reactions using primers specific to an LMW glutenin gene were performed for Harunoakebono, the NIL and Glenlea and amplified a novel LMW-s glutenin gene for Glenlea and the NIL and another LMW-s glutenin gene for Harunoakebono. Genomic PCRs using primers designed on the basis of internal sequences of the genes, s-F1/s-R2, were performed for the segregating population and amplified two DNA fragments that correspond to the LMW-s glutenin genes in reverse transcription-polymerase chain reaction and are, respectively, linked to LMW glutenin components from Glenlea associated with good bread-making quality and the allelic components from Harunoakebono. The results suggest that a novel LMW-s glutenin gene encodes LMW glutenin components associated with good bread-making quality.

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http://www.sciencedirect.com/science/article/B6T02-4031TYD-8/2/8567071b43de4653bd9dae2bcfde1333

An affinity-purified anti-peptide antibody generated against the carboxy-terminal region of the [delta] opioid receptor was used to localize [delta] opioid receptors in mouse brain. [delta] Opioid receptor immunoreactivity was found in axons and nerve terminals in regions of the olfactory bulb, hippocampal formation, cerebral and cerebellar cortex, midbrain and hindbrain. The immunocytochemical distribution correlated well, though not completely with autoradiographic distribution of [delta] opioid receptors in mouse brain using either [3H][2--penicillamine, 5--penicillamine]-enkephalin (DPDPE) or [3H]naltrindole. Confocal microscopy of double-labeled tissue provided direct evidence that [delta] opioid receptors are principally expressed on GABAergic terminals in the hippocampus. These anatomical findings complement extensive physiological studies to provide a more detailed description of endogenous opioid circuitry.


http://www.sciencedirect.com/science/article/B6T02-429Y5BB-7/2/119d8d4a9815fa1f124dcb8153ef6c3e

Expression of the noradrenaline transporter (NAT) was identified in various cell and fibre populations of the rat adrenal medulla, examined with immunohistochemistry and confocal microscopy. Immunoreactivity for the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), aromatic–amino-acid decarboxylase (AADC) and dopamine [beta]-hydroxylase (DBH) was present in all chromaffin cells, while phenylethanolamine N-methyltransferase (PNMT) was used to determine adrenergic chromaffin cell groups. Labelling with NAT antibody was predominantly
cytoplasmic and colocalised with PNMT immunoreactivity. Noradrenergic chromaffin cells were not NAT immunoreactive. Additionally, NAT antibody labelling demonstrated clusters of ganglion cells (presumably Type I) and nerve fibres. Expression of TH, AADC, DBH, PNMT and NAT mRNA was examined using reverse transcription-polymerase chain reaction (RT-PCR) from adrenal medulla punches and single chromaffin cells, and results were consistent with those obtained with immunocytochemistry. Chromaffin cells and fibres labelled with antibodies against growth associated protein-43 (GAP-43) were not NAT immunoreactive, while ganglion cells were doubled labelled with the two antibodies. The presence of NAT in adrenergic chromaffin cells, and its absence from noradrenergic cells, suggests that the adrenergic cell type is primarily responsible for uptake of catecholamines in the adrenal medulla.


Spinal cord injury (SCI) is associated with progressive neurodegeneration and dysfunction. Multiple cellular and molecular mechanisms are involved in this pathogenesis. In particular, the activation of proteases following trauma can cause apoptosis in the spinal cord. Calpain, a calcium-dependent cysteine protease, plays a major role in apoptosis following trauma. We identified apoptosis and decrease in transcription of the genes for proteolipid protein (PLP) and myelin basic protein (MBP) in five 1-cm long spinal cord segments (S1, distant rostral; S2, near rostral; S3, lesion; S4, near caudal; and S5, distant caudal) 24 h after induction of SCI (40 g cm$^{-1}$ force) in rats by weight-drop method. Sham rats underwent laminectomy and did not receive injury. Internucleosomal DNA fragmentation occurred prominently in the lesion (S3), moderately in near segments (S2 and S4), and slightly in distant segments (S1 and S5) of injured rats, indicating the occurrence of apoptosis in the lesion and penumbra. Levels of transcription of PLP and MBP were reduced highly in the lesion and moderately in near segments, suggesting that apoptotic loss of cells impaired biosynthesis of two important structural components of myelin. Immediate administration of the calpain inhibitor E-64-d (1 mg/kg) to injured rats prevented apoptosis and restored transcription of these genes, indicating the therapeutic efficacy of calpain inhibitor for treatment of SCI.


Analysis of single-strand conformation polymorphism (SSCP) by capillary electrophoresis (CE) was developed. The conformational change of single-strand DNA is caused by a mutation in a DNA fragment. The change is detected as mobility shift in CE. The effects of acrylamide gel concentration, running temperature and fragment size amplified by the polymerase chain reaction
(PCR) were studied to develop the separation of SSCP. The model DNA used was the divE 42 gene carrying wild- and mutant-type (G→A point mutation at the 141 site). The results show that two single-strand DNA fragments that differ in one nucleotide can be separated by CE within minutes. This method was also applied to the separation of SSCP for N-ras gene including four kinds of mutations. All mutations tested in this study could be distinguished. CE is well suited for clinical analysis of SSCP because it is rapid and reproducible, allows on-line detection and is easy.


http://www.sciencedirect.com/science/article/B6TG8-44CPVGG-VF/2/9104831ae627cefb4b8c28e59bab3d2c

Capillary gel electrophoresis (CGE) was studied for the direct analysis of polymerase chain reaction (PCR) amplified samples. A low cross-linked polyacrylamide gel (3%T, 0.5%C) was used for CGE with treated and untreated silica capillaries. CGE showed high reproducibility and resolution in the separation of DNA fragments (ca. 100-1000 base pairs) produced by PCR. The CGE system was applied to the detection of an amplification refractory mutation system (ARMS) and PCR-restriction fragment length polymorphism (PCR-RFLP), which are detection methods of single base substitution in genes using PCR. With the CGE system, full automation of PCR product detection is feasible.


http://www.sciencedirect.com/science/article/B6TG8-44CP9S94-50/2/e05a0e94df4fc24ccd7db73db19d223

Capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF) has been developed to detect polymerase chain reaction (PCR) amplified samples. LIF detection was performed using Thiazole Orange as the fluorescent intercalating dye. This method was ca. 100X as sensitive as that with UV detection. The highly sensitive CGE-LIF was applied to the detection of the most prevalent mutation (lysine329- to-glutamic acid substitution) in medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency. The disorder, which shows an autosomal recessive inheritance, is known to be highly prevalent among Caucasian population and often mimics as Reye-like syndrome or sudden infant death. A DNA fragment containing the mutation site was PCR-amplified with two sets of allele specific oligonucleotide primers, followed by CGE-LIF. The mutant allele produced a 175-base pairs DNA fragment, which the normal allele generated a 202-base pairs DNA fragment. CGE-LIF clearly distinguished these PCR products, facilitating rapid diagnosis of MCAD deficiency.

A deoxyribonuclease bioreactor was prepared by immobilization of deoxyribonuclease I through epoxy groups inherently present on poly (glycidyl methacrylate-co-ethylene dimethacrylate) monoliths. Columns with various levels of DNase activity were prepared varying immobilization temperature, pH, time and method. The apparent Michaelis-Menten constant, and turnover number, for immobilized DNase determined by on-line frontal analysis method were, respectively, 0.28 g of DNA l-1 and 16 dA260nm min-1 mg-1 of immobilized DNase. The highest activity of immobilized DNase was detected at 1 mM calcium ions concentration and mirrored properties of free enzyme; however, reaction temperature in the range from 25 to 37 [deg]C has no significant effect on activity of immobilized DNase in contrary to free enzyme. The CIM DNase bioreactor was used for elimination of DNA contaminants in RNA samples prior to reverse transcription followed by PCR.


The viral safety of plasma-derived products with respect to hepatitis C virus (HCV) is assured by selection of donors, screening of individual donations for antibodies to HCV and the incorporation of effective viral inactivation-removal steps into manufacturing processes. As antibody screening of single donations is not sufficient to completely eliminate HCV RNA positive plasmas from plasma pools, testing for HCV RNA by gene amplification techniques may be necessary to identify positive donations. Using modern molecular biology techniques, we developed a specific, sensitive and reproducible method for routine PCR screening for HCV RNA in plasma pools.


Detection and quantitation of gene expression in single cells is especially important in the central nervous system where, at the cellular level, the synapse can be considered the single functional unit. For example, the consolidation of long-term memories may be mediated by persistent changes in the strength of synaptic transmission at individual synapses. In order to investigate the requirement for de novo RNA synthesis during long-term potentiation in individual neurons, we have combined single-cell electrophysiology with single-cell gene-expression methodology. Described are methods combining whole-cell patch-clamp and single-cell RT-PCR for the detection of a single mRNA species for nitric oxide synthase, or, through a multiplex strategy, for the simultaneous detection of several mRNAs including heme oxygenase 2, protein phosphatase inhibitor 1 protein, and several isoforms of the calcium/calmodulin dependent protein kinase II.

The sizing capability of slab gel electrophoresis for short tandem repeat (STR) fragments was compared to the sizing capability of capillary electrophoresis (CE). Both systems used automated laser fluorescence detection to detect four fluorescent dyes, enabling the use of an internal lane standard within each sample. The STR fragments were amplified using a multiplex polymerase chain reaction (PCR) in which the STR fragments Hum CD-4, Hum TH01, Hum D21S11 and Hum SE33 were amplified simultaneously. The reproducibility of the size calling was determined for both systems. The average standard deviation obtained for the slab gel system was 0.2, which was comparable to the standard deviation of 0.12 obtained for the CE system. The CE system produced results comparable to those obtained on the slab gel system, with a level of precision of +/-1.0 bp (between instruments).


Capillary electrophoresis (CE) was used to characterize restriction fragment length polymorphism (RFLP) in a polymerase chain reaction (PCR)-amplified product of a 740-base pairs DNA fragment from the DXS 164 locus of the dystrophin gene. The polymorphic alleles of 740 and 520/220 base pairs revealed by XmnI digestion were analysed from homozygous and heterozygous individuals by CE. Our studies show that extraction in phenol-chloroform may be useful in PCR-amplified product purification. Excellent separation was obtained in a short time. The data indicate that CE is suitable for genomic analysis such as carrier detection and prenatal diagnosis of X-linked recessive disorders after purification of PCR-amplified products.


We demonstrated fast DNA separations in low viscosity entangled solutions with a temperature gradient in a non-denaturing separation medium. The separations were carried out in solution of commercially available poly(ethylene oxide) (PEO) [1 x Tris(hydroxymethyl)aminomethane borate buffer, without urea] with a temperature gradient of 2[deg]C/min. The performance was compared with that of a solution of PEO with urea at ambient temperature. We found that the former condition gives sufficient resolution for accurate base calling and that in general, it gave better separation for fragments larger than 450 base pairs (bp). Most importantly, the separation speed approaches 30 bp/min. In addition, we describe a simple yet reliable gel preparation protocol for such separations.

DNA sequencing from sub-microliter samples was demonstrated for capillary array electrophoresis by optimizing the analysis of 500 nl reaction aliquots of full-volume reactions and by preparing 500 nl reactions within fused-silica capillaries. Sub-microliter aliquots were removed from the pooled reaction products of 10 [μl] dye-primer cycle-sequencing reactions and analyzed without modifying either the reagent concentrations or instrument workflow. The impact of precipitation methods, resuspension buffers, and injection times on electrokinetic injection efficiency for 500 nl aliquots were determined by peak heights, signal-to-noise ratios, and changes in base-called readlengths. For 500 nl aliquots diluted to 5 [μl] in 60% formamide-1 mM EDTA and directly injected, a five-fold increase in signal-to-noise ratios was obtained by increasing injection times from 10 to 80 s without a corresponding increase in peak widths or reduction in readlengths. For 500 nl aliquots precipitated in alcohol, 80+/−5% template recovery and a two-fold decrease in conductivity was obtained, resulting in a two-fold increase in peak heights and 50 to 100 bases increase in readlengths. In a comparison of aliquot volumes and precipitation methods, equivalent readlengths were obtained for 500 nl, 4 [μl], and 8 [μl] aliquots by simply adjusting the electrokinetic injection conditions. To ascertain the robustness of this methodology for genomic sequencing, 96 Arabidopsis thaliana subclones were sequenced, with a yield of 38624 bases obtained from 500 nl aliquots versus 30764 bases from standard scale reactions. To demonstrate 500 nl sample preparation, reactions were performed in fused-silica capillary reaction chambers using air-based thermal cycling. A readlength of 690 bases was obtained for the polymerase chain reaction product of an Arabidopsis subclone without modifying the reagent concentrations, post-reaction processing or electrokinetic injection workflow. These results demonstrated the fundamental feasibility of small-volume DNA sequencing for high-throughput capillary electrophoresis.


The effect of the electric field strength gradient on the separation of DNA sequencing fragments was investigated. We demonstrate that the stepwise gradient of electric field improves the separation of DNA sequencing fragments more than 500 bases in size and diminishes the analysis time for DNA sequencing of lager DNA fragments. The use of the electric field strength gradient induces an increase in the theoretical plate number as predicted by the theoretical formulation discussed in this paper.


Mutation of the p53 gene plays an important role in neoplastic progression in human tumorigenesis. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) techniques are now available for the detection of point mutations. The original method
using polyacrylamide gel electrophoresis is disadvantageous, particularly for clinical tests and for analysis of large numbers of samples. Therefore, using an automated capillary electrophoresis (CE) technique with a molecular-sieving polymer solution, we have devised a completely automatic fluorescence-based PCR-SSCP system (CE-FSSCP) for the differential detection of point mutations that do not require SSCP with radioisotopes and polyacrylamide gels. This automatic CE-FSSCP system was developed for reproducible operations in the denaturation of double-stranded DNA and electrophoresis of single-stranded DNA. The detection system consists of a 100 W I2 lamp and photomultiplier. We performed CE-FSSCP with a 2% linear polyacrylamide polymer solution containing 5% glycerol. Four tissue specimens of lung tumors with mutations in exon 7 of the p53 gene were found to have mutant alleles; a six-base-pair deletion at codons 247-248, a one-base-pair deletion at codon 260, a one-base-pair deletion at codon 244 and a GGC to CGC substitution at codon 244. We expect this technique to prove useful for the clinical DNA diagnosis of human cancers, determination of the therapeutic effect of anticancer agents and for the study of the molecular aspects of the mechanisms involved in the pathogenesis of human cancers.


http://www.sciencedirect.com/science/article/B6TG8-3SDKTD6-17/2/0966f83ca92c48ae0b0634fbdeda55d3ce

We have demonstrated that DNA bases up to 1000 base pairs (bp) in a sequencing ladder can be separated using poly(ethylene oxide)-filled capillary electrophoresis (resolution of raw data=0.5 at 966 bp). Separation performance of this sieving matrix has been tested under different experimental conditions. It was found that the electric field strength played a critical role in the onset of reptation and thus the separation efficiency. Optimized gel composition and concentration is required for good separation, but the total gel concentration should lie between 2.5 and 3.0%. We observed that the capillary length influences the number of theoretical plates and the maximum readable length of DNA. For sequencing up to 500 bp, relatively nonviscous solutions can be used, greatly facilitating the replacement of the sieving matrix in between runs.


http://www.sciencedirect.com/science/article/B6TG8-3SDKTD6-1D/2/8a8a6d6bfd0150bb895b3a2283323aba

Capillary electrophoresis (CE) with laser-induced fluorescence (LIF) was used to detect known point mutations using the method of single-nucleotide primer extension (SNUPE). Three different point mutations in human mitochondrial DNA associated with Leber's hereditary optic neuropathy (LHON) were detected by annealing a primer immediately 5' to the mutation on the template and extending the primer by one fluorescently labeled deoxy terminator complementary to the mutation. By using two or more differently labeled terminators, both the mutant and wild type could be simultaneously detected. The advantages of using CE-LIF for detecting SNUPE reactions include speed and ease of analysis, absence of radioactivity, and potential for automation.

The possibility of using polymer mixtures with different chemical compositions as a DNA sequencing matrix by capillary electrophoresis (CE) has been exploited. Polyacrylamide (PAM, 2.5%, w/v) having a molecular mass of 2.2.10^6 has been mixed with poly(N,N-dimethylacrylamide) (PDMA) having molecular masses of 8000, 470000 and 2.1.10^6 at concentrations of 0.2, 0.5 and 1% (w/v). Unlike polymer mixtures of the same polymer with different molecular masses, the use of polymer mixtures with different chemical compositions encounters an incompatibility problem. It was found that the incompatibility increased with increasing PDMA molecular mass and PDMA concentration, which resulted in decreased efficiency in DNA sequencing. Also, the incompatibility had a more pronounced effect on the efficiency as the base number was increased. However, by choosing a low-molecular-mass PDMA of 8000 and a low concentration of 0.2% (w/v), the incompatibility of PAM and PDMA has been alleviated. At the same time, the advantage of using polymer mixtures revealed a higher efficiency for such a polymer mixture when compared with PAM. The mixture also endowed the separation medium with a dynamic coating ability. An efficiency of over 10.10^6 theoretical plates per meter has been achieved by using the bare capillaries without the additional chemical coating step.


We are currently developing miniaturized, chip-based electrophoresis devices fabricated in plastics for the high-speed separation of oligonucleotides. One of the principal advantages associated with these devices is their small sample requirements, typically in the nanoliter to sub-nanoliter range. Unfortunately, most standard sample preparation protocols, especially for oligonucleotides, are done off-chip on a microliter-scale. Our work has focused on the development of capillary nanoreactors coupled to micro-separation platforms, such as micro-electrophoresis chips, for the preparation of sequencing ladders and also polymerase chain reactions (PCRs). These nanoreactors consist of fused-silica capillary tubes (10-20 cm x 20-50 [mu]m I.D.) with fluid pumping accomplished using the electroosmotic flow generated by the tubes. These reactors were situated in fast thermal cyclers to perform cycle sequencing or PCR amplification of the DNAs. The reactors could be interfaced to either a micro-electrophoresis chips via capillary connectors micromachined in polymethylmethacrylate (PMMA) using deep X-ray etching (width 50 [mu]m; depth 50 [mu]m) or conventional capillary gel tubes using zero-dead volume glass unions. For our chips, they also contained an injector, separation channel (length 6 cm; width 30 [mu]m; depth 50 [mu]m) and a dual fiber optic, near-infrared fluorescence detector. The sequencing nanoreactor used surface immobilized templates attached to the wall via a biotin-streptavidin-biotin linkage. Sequencing tracks could be directly injected into gel-filled capillary tubes with minimal degradation in the efficiency of the separation process. The nanoreactor could also be configured to perform PCR reactions by filling the capillary tube with the PCR reagents and template. After thermal cycling, the PCR cocktail could be pooled from multiple reactors and loaded onto a slab gel or injected into a capillary tube or microchip device for fractionation.

http://www.sciencedirect.com/science/article/B6X0P-46X8XN2-3/2/14c7cb7608dc310e9f52efab581dac97

Alpha–fucosidase (FUC) is a glycosidase involved in the degradation of fucose-containing glycoconjugates. A cDNA representing the complete sequence of human FUC was inserted into the prokaryotic expression vector pGEX-2T. High levels of the glutathione S-transferase (GST) fusion protein were detected in Escherichia coli cells after induction with isopropyl thio-beta-galactopyranoside. The GST-FUC protein was mostly found as inclusion bodies and attempts to optimise its expression as a soluble form were unsuccessful. Nevertheless, the recombinant protein was purified by affinity chromatography on glutathione-sepharose and its fucosidase activity was characterised. After thrombin cleavage of the GST tag, the FUC precursor protein was purified by electro-elution.


http://www.sciencedirect.com/science/article/B6X0P-46FMKN4-2/2/05a178a664df754b1fca3f12fe848ee8

The extent of the DNA methylation of genomic DNA as well as the methylation pattern of many gene-regulatory areas are important aspects with regard to the state of genetic information, especially their expression. There is growing evidence that aberrant methylation is associated with many serious pathological consequences. As genetic research advances, many different approaches have been employed to determine the overall level of DNA methylation in a genome or to reveal the methylation state of particular nucleotide residues, starting from semiquantitative methods up to new and powerful techniques. In this paper, the currently employed techniques are reviewed both from the point of view of their relevance in genomic research and of their analytical application. The methods discussed include approaches based on chromatographic separation (thin-layer chromatography, high-performance liquid chromatography, affinity chromatography), separation in an electric field (capillary electrophoresis, gel electrophoresis in combination with methylation-sensitive restriction enzymes and/or specific sequencing protocols), and some other methodological procedures (mass spectrometry, methyl accepting capacity assay and immunoassays).


http://www.sciencedirect.com/science/article/B6X0P-49679S6-3/2/1cbbf7a1b8714ecbf56602e73163cb9d
To explore if it is correlated in human tumor cells that the expression of LDH homologous gene and LDH isoenzymes, we used RT-PCR-SSCP technique to measure the relative expression of genes with homologous sequences. The combination of PCR using common primers designed in the highly conserved regions and single-strand conformation polymorphism analysis of the products is used for quantitative determination of the proportions of LDH-A mRNA in human cancer cell lines. The proportion is compared with that of the activities of isoenzymes. The results indicated that the enzyme activity of LDH-A was consistent with mRNA levels in the human tumor cell. The present procedure using a single pair of primers for two fragments can overcome disadvantages in quantitative analysis using multiplex PCR. Template concentrations and PCR cycles did not affect the proportions of LDH-A and LDH-B in the product.


http://www.sciencedirect.com/science/article/B6X0P-46W1CRX-C/2/a6fba080eda63a1a5c9ddbf6705a6c2

Incorporation of non-complementary nucleotides during polymerase chain reaction can result in ambiguous denaturing high-performance liquid chromatography profiles that reduce both sensitivity and specificity of mutation analysis. The use of proofreading DNA polymerases increases the fidelity of polymerase chain reaction and, consequently, reduces background noise in the chromatograms. This is demonstrated for several BRCA1 and BRCA2 mutations hat had yielded previously chromatograms of poor quality using non-proofreading enzyme for amplification. Interestingly, despite the reduced level of background heteroduplexes, the ability of denaturing high-performance liquid chromatography to detect mutant alleles at a frequency <10% in pools of chromosomes did not improve significantly.


http://www.sciencedirect.com/science/article/B6X0P-470M7NT-5/2/8eaa2ae4fc0066683bbf4of474bd9c8d

The introduction of alkylated, nonporous poly-(styrene-divinylbenzene) microparticles in 1992 enabled the subsequent development of denaturing HPLC that has emerged as the most sensitive screening method for mutations to date. Denaturing HPLC has provided unprecedented insight into human origins and prehistoric migrations, accelerated the cloning of genes involved in mono- and polygenic traits, and facilitated the mutational analysis of more than a hundred candidate genes of human disease. A significant step toward increased sample-throughput and information content was accomplished by the recent introduction of monolithic poly(styrene-divinylbenzene) capillary columns. They have enabled the construction of capillary arrays amenable to multiplex analysis of fluorescent dye-labeled nucleic acids by laser-induced fluorescence detection. Hyphenation of denaturing HPLC with electrospray ionization mass spectrometry, on the other hand, has allowed the direct elucidation of the chemical nature of DNA variation and determination of phase of multiple alleles on a chromosome.

Optimization of electrophoretic techniques is becoming an increasingly important area of research as microdevices are now routinely adapted for numerous biology and engineering applications. The present work seeks to optimize electrophoresis within microdevices by utilizing ultra-high voltages to increase sample concentration prior to separation. By imaging fluorescently-tagged DNA samples, the effects of both conventional and atypical voltage protocols on DNA migration and separation are readily observed. Experiments illustrate that short periods of high voltage during electrophoretic injection do not destroy the quality of DNA separations, and in fact can enhance sample concentration five-fold. This study presents data that illustrate increases in average resolution, and resolution of longer fragments, obtained from electrophoretic injections utilizing voltages between 85 and 850 V/cm.


Accurate and fast genotyping of single nucleotide polymorphisms (SNPs) is important in the human genome project. Here an automated fluorescent method that can rapidly and accurately genotype multiplex known SNPs was developed by using a homemade kit, which has lower cost but higher resolution than commercial kit. With this method, oncogene K-ras was investigated, four known SNPs of K-ras gene exon 1 in 31 colorectal cancer patients were detected. Results indicate that mutations were present in 8(26%) of 31 patients, and most mutations were localized in codon 12. The presence of these mutations is thought to be a critical step and plays an important role in human colorectal carcinogenesis.


We describe the development of a sensitive high-performance liquid chromatographic (HPLC) method for polymerase chain reaction (PCR) products using bisbenzimide (Hoechst 33258 dye) based fluorimetric detection. The detection limit and specificity for double-strand DNA detection are improved in comparison with HPLC with UV absorbance detection. This HPLC, using a column packed with diethylaminoethyl-bonded non-porous resin particles, was applied to the detection of allele-specific PCR and restriction fragment length polymorphism analysis. We also developed a hybridization method analyzed by HPLC. DNA fragments (149 bp) containing the mutation site (C->A,G,T) in the N-ras gene were amplified by PCR. Fluorescein isothiocyanate (FITC)-labeled DNA probes were also prepared by PCR using FITC-labeled 5' primer. Analysis of
mutation was performed by the separation of a hybrid and non-reactive DNA probe with HPLC with fluorimetric detection after the hybridization of target DNA (149 bp) and a FITC DNA probe. The effects of various factors on hybridization were examined to establish optimal assay conditions. Under the conditions determined, a point mutation in PCR products obtained from the N-ras gene could be detected specifically by this method. The analysis of PCR products by HPLC may potentially be useful for DNA diagnosis.


http://www.sciencedirect.com/science/article/B6TG9-3V9DKTF-D/2/7bef7f875bfae2ff4ad1577af1e715e8

The analysis of crude polymerase chain reaction (PCR) products by capillary electrophoresis (CE) is often compromised due to the presence of a high concentration of salt. Salt interferes with the electrokinetic injection and induces localized heating within the column; hence, PCR products must be desalted or cleaned-up prior to CE analysis. A variety of commercial clean-up systems are available that have been traditionally used to prepare PCR products for cloning, sequencing and digestion with restriction enzymes. These systems were tested for their effectiveness in preparing PCR products for CE analysis and were evaluated based on CE resolution, salt removal, DNA recovery, processing time and cost. One particularly effective clean-up system, membrane dialysis, was automated using a robotic workstation.


http://www.sciencedirect.com/science/article/B6TG9-457CV9F-NK/2/f89b7ab0576841826e0a63b0f0aed563

In samples where the amount of DNA is limited, the polymerase chain reaction (PCR) can amplify specific regions of the DNA. A quantitative analysis of the PCR product would be desirable to ensure sufficient DNA is available for analysis. In this study, we examine the use of capillary electrophoresis (CE) with laser fluorescence detection for quantitation of PCR products. A coated open tubular capillary was used with a non-gel sieving buffer and a fluorescent intercalating dye to obtain results within 20 minutes. Using an internal standard, peak migration time was below 0.1% relative standard deviation (R.S.D.) with a peak area precision of 3% R.S.D. In comparison to quantitation by hybridization, (i.e., slot blot) and spectrophotometric analysis, capillary electrophoresis shows distinct advantages due to its ability to separate unincorporated primers and PCR byproducts from the targeted PCR product. The results demonstrate that CE can be used to monitor the quality and quantity of the PCR product.


http://www.sciencedirect.com/science/article/B6TG9-4037929-2/2/a6327c53460a46ed95d6199ac7a1123d
Current high-throughput approaches to the analysis of PCR products are based primarily on electrophoretic separation and laser-excited fluorescence detection. We show that capillary array electrophoresis can be applied to HIV-1 diagnosis and D1S80 VNTR genetic typing based simply on UV absorption detection. The additive contribution of each base pair to the total absorption signal provides adequate detection sensitivity for analyzing most PCR products. Not only is the use of specialized and potentially toxic fluorescent labels eliminated, but also the complexity and cost of the instrumentation are greatly reduced.


http://www.sciencedirect.com/science/article/B6TG9-42MFDJP-13/2/db2c17567a9a8d1668d6deda412f5c53

High-performance liquid chromatography (HPLC) has been applied to the multiplex detection of the two single nucleotide mutations commonly found in hereditary hemochromatosis (HH). HH is associated with a major G to A transition at position 845 (mutation Cys282Tyr) and a minor C to G transition at position 187 (mutation His63Asp) in the cDNA of the HFE gene. Two detection assays were developed based on HPLC analysis of restriction fragment length polymorphism (RFLP) or single nucleotide extension (SNE) products following multiplex PCR amplification. RFLP genotypes the two sites as dsDNA fragments of different lengths generated by restriction enzymes Rsa I/Bcl I. SNE extends primers 5'-adjacent to the sites of interest with a dideoxynucleotide triphosphate (ddNTP) to generate extended ssDNA. The identity of the added ddNTP reveals the identity of the original possible mutation site(s). Application of these methods with HPLC analysis provides simple and reliable genotyping for HH and can be applied to other single nucleotide polymorphism studies.


http://www.sciencedirect.com/science/article/B6TG9-4002HC1-8F/2/2effe9bccf4578aa7f110e5556c35504

The experiments described in the present paper were performed in order to determine whether the Biomek-1000 (Beckman Instruments, Fullerton, CA, USA) automated laboratory workstation can be used in a fully automated DNA labeling method followed by automated gravity-driven size exclusion purification of molecular probes. To this aim, we performed random oligodeoxyribonucleotide priming of a HIV-1 LTR probe that was used for molecular hybridization to Southern blotted polymerase chain reaction products. The results obtained demonstrate that the automatically labeled probe can be efficiently purified by automated and gravity-driven Sephadex G-50 chromatography, without any major changes in hybridization property. This robotic methodology can be used in several procedures employing radioisotope labeling.

This study dealt with the application of capillary gel electrophoresis (CGE) to diagnosis of the aldehyde dehydrogenase 2 (ALDH-2) genotype. Electrophoresis was performed on a low cross-linked polyacrylamide gel (3% T [g acrylamide + g Bis (N,N'-methylenebisacrylamide)], 0.5% C (g Bis/%T)) in 100 mM Tris-borate buffer (pH 8.3) at -10 KV with on-column UV detection (260 nm). During the PCR reaction, DNA from the wild-type allele generated a MboII restriction site, which is an amplification created restriction site. This did not occur, however, with DNA fragments from the mutant allele. Therefore, determination of the heterozygous genotype, the coexistence of wild-type and mutant alleles, was easily possible. Analysis of the MboII restriction digests of the PCR products was completed in less than 20 min, showing two peaks corresponding to fragments of 125 (cleaved) and 135 (uncleaved) base pairs (bp), respectively. On the other hand, determination of the homozygous genotype, wild-type or mutant, was difficult in one electrophoresis run. The CGE of the MboII restriction digests gave a single peak and the identification, cleaved or uncleaved, was difficult under our experimental conditions. However, the addition of aliquots of the PCR reaction mixture to the restriction digests, followed by re-electrophoresis, allowed successful diagnosis, yielding two peaks (cleaved and uncleaved) for the wild-type and one peak (uncleaved) for the mutant allele. This study demonstrated that CGE offers a high-speed, high-resolution analytical tool for determining genetic types, as compared with the conventional slab gel methodologies.


A reliable protocol was designed for fast expression and purification of recombinant chymotrypsin(ogen). The zymogen was overexpressed in soluble form as a (His)6-fusion construct in the cytoplasm of the thioredoxin reductase deficient Escherichia coli strain AD494(DE3). This allowed purification of chymotrypsinogen in a highly selective affinity chromatography capture step using a Ni-NTA column. After activation with enterokinase, the enzymatically active chymotrypsin was purified in a polishing step using a modified soybean trypsin inhibitor agarose column. This expression system and the use of affinity chromatography for capture and polishing, offers an easier and faster route to recombinant chymotrypsin(ogen) than the previously described use of Saccharomyces cerevisiae.

speed separation and good resolution can be fulfilled by using a single-MW PEO polymer. It offers similar separation performance as before for the small DNA fragments, but better performance for large DNA fragments.

Journal of Clinical Forensic Medicine (1)


In poisoning, detection of the nature of causative agent is important for management of trauma and forensic investigation. Most of the methods in clinical toxicology are developed for detection of toxins and poisons. A random amplified polymorphic DNA (RAPD)-based method has been described for detection of species of animal from its morphologically unrecognizable fragments, recovered from food substances, consumption of which caused even death. Pre-mixed RAPD reaction beads and six RAPD primers were used in polymerase chain reaction analysis. Among six RAPD primers used, any one of them was sufficient in resolving this practical forensic situation. To enhance the probability values for matching in the present study of fixing identity of an animal, six set of market available RAPD primers were used. This is the first report of a forensic application of RAPD DNA typing in identification of charred skeleton remnants of Lizard species in food material. Furthermore unique amplicons were generated for different reptilian species, which can be used as species specific markers for species identification in forensic situation, however, no variations among individuals of same species were observed.

Journal of Clinical Microbiology (2)


In a recent study, immunoglobulin G in human plasma was identified as a major inhibitor of diagnostic PCR (W. Abu Al-Soud, L. J. Jonsson, and P. Radstrom. J. Clin. Microbiol. 38:345-350, 2000). In this study, two major PCR inhibitors in human blood cells were purified using size exclusion and anion-exchange chromatographic procedures. Based on N-terminal amino acid sequencing and electrophoretic analysis of the purified polypeptides, hemoglobin and lactoferrin were identified as PCR-inhibitor components in erythrocytes and leukocytes, respectively. When different concentrations of hemoglobin or lactoferrin were added to PCR mixtures of 25 {micro}l containing 10 different thermostable DNA polymerases and 1 ng of Listeria monocytogenes DNA as template DNA, AmpliTaq Gold, Pwo, and Ultima were inhibited in the presence of [\&lt;=]1.3 {micro}g of hemoglobin and [\&lt;=]25 ng of lactoferrin, while rTth and Tli were found to resist inhibition of at least 100 {micro}g of hemoglobin. In addition, the quantitative effects of seven low-
molecular-mass inhibitors, present in blood samples or degradation products of hemoglobin, on real-time DNA synthesis of rTth using the LightCycler Instrument were investigated. A reaction system based on a single-stranded poly(dA) template with an oligo(dT) primer annealed to the 3' end was used. It was found that the addition of 0.25 to 0.1 mg of bile per ml, 2.5 mM CaCl₂, 0.25 mM EDTA, 5 μM FeCl₃, and 0.01 IU of heparin per ml reduced the fluorescence to approximately 76, 70, 46, 17, and 51%, respectively. Finally, the effects of nine amplification facilitators were studied in the presence of hemoglobin and lactoferrin. Bovine serum albumin (BSA) was the most efficient amplification facilitator, so that the addition of 0.4% (wt/vol) BSA allowed AmpliTaq Gold to amplify DNA in the presence of 20 instead of 1 μg of hemoglobin and 500 instead of 5 ng of lactoferrin. Including 0.02% (wt/vol) gp32, a single-stranded-DNA binding protein, in the reaction mixture of AmpliTaq Gold was also found to reduce the inhibitory effects of hemoglobin and lactoferrin.


http://jcm.asm.org/cgi/content/abstract/38/1/125

The multiplex PCR method for the detection of Alloiococcus otitidis, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae (P. H. Hendolin, A. Markkanen, J. Ylikoski, and J. J. Wahlfors, J. Clin. Microbiol. 35:2854-2858, 1997) in middle ear effusions (MEEs) was modified to be better suited for clinical use. To detect false-negative results, an internal amplification was added to the reaction, and to prevent carryover contamination, the dUTP-uracil-N-glycosidase system was incorporated into the procedure. Labor was minimized by using the heat-activatable AmpliTaq Gold polymerase in order to circumvent manual hot start and by detecting the amplification products on an automated sequencer. The performance of the improved protocol was verified with MEEs from patients with otitis media with effusion. In addition, a ligase detection reaction (LDR) was developed for confirmation of the PCR products. The modifications increased the reliability of the protocol and the hands-off time significantly. However, when two DNA extraction protocols were compared, gram-negative bacteria were detected more often in phenol-treated MEEs (94 versus 46%; P < 0.001), and gram-positive bacteria were detected more often in MEEs dissolved in sodium dodecyl sulfate-NaOH-chaotropic salt (83 versus 27%; P < 0.001). The LDR was found to be 100% specific. In all, the results demonstrate the feasibility of the rapid (7-h) multiplex PCR method for routine laboratory use.

Journal of Comparative Pathology (2)


http://www.sciencedirect.com/science/article/B6WHW-4F6CRBN-3/2/1f5a8a0efb70018e602710da697325c7

SummaryCanine pigmented epidermal nevus (CPEN) is a skin disorder of some breeds of dog characterized by multiple black plaques of the haired and non-haired skin. Three cases of pigmented cutaneous papillomatosis (previously described also as CPEN) in pug dogs were
investigated histopathologically, immunohistochemically and electron microscopically. Additionally, DNA analyses with the polymerase chain reaction (PCR) were performed in two cases. Many nuclei of the stratum granulosa were diffusely immunolabelled for specific structural antigens of bovine papillomavirus (subgroup A), but nuclear inclusion bodies were not detected by retrospective examination of haematoxylin and eosin-stained sections of the affected skin. Aggregates of small numbers of viral particles (ranging from 37 to 43 nm in diameter) with a hexagonal structure were sparsely scattered throughout the nuclei of some of the superficial keratinocytes. PCR amplification targeted for the L1 gene of papillomavirus cloned from a case of CPEN yielded an expected fragment of 194-bp in the two CPEN cases examined but not in a case of canine oral papilloma.


http://www.sciencedirect.com/science/article/B6WHW-4CPM121-1/2/b38125619b296ea4fc709c8aab578a7b

Interstitial lung disease with chronic fibrosis is a frequent cause of reduced performance in horses. The aim of this study was to establish a model of acute alveolar damage and interstitial lung disease in horses that could be used to monitor the histopathological lesions and changes in expression levels of genes relevant to pulmonary fibrosis. Six adult horses were given a single intravenous injection (6 mg per kg body weight) of perilla mint ketone (PMK). Transthoracic lung biopsy samples (1 x 0.2 x 0.2 cm) were collected before and after (days 1, 4, 8, 11, 15, 18, 22, 25 and 29) the administration of PMK. Light and electron microscopy revealed severe acute alveolar damage (days 1 to 4), proliferation of type II pneumocytes (days 4 to 11) and finally complete healing at about day 18. However, unexpectedly severe clinical signs necessitated euthanasia in two horses on days 9 and 11. The expression levels of the collagen genes COL1AI and COL3AI as well as transforming growth factor (TGF)-[beta] were examined in the biopsy samples by reverse transcription-real time quantitative polymerase chain reaction. COL1AI and COL3AI gene expressions were upregulated (3- and 17-fold, respectively) between days 1 and 29 in all six horses, whereas TGF-[beta] was upregulated in two horses (2- and 4-fold, respectively), between days 4 and 18. Although the gene expression analyses indicated a strong activation of the pro-fibrotic pathway, no interstitial fibrosis was seen in any horse. A complete necropsy performed on day 60 revealed complete recovery of the lungs of the four surviving horses, with no evidence of fibrosis. Unidentified compensatory mechanisms may have prevented pulmonary fibrosis, despite strong upregulation of pro-fibrotic genes.

Journal of Cystic Fibrosis (1)


http://www.sciencedirect.com/science/article/B6X2D-4CSG1X7-1/2/f47450de20d468e83c5bf97211802381

The scope of this article is to outline some of the basic methods for good quality RNA preparation
from mammalian tissues and cells (including epithelial cells). Additionally, we give an outline of common techniques of measuring CFTR gene expression such as quantitative and semi-quantitative reverse transcription (RT) PCR and ribonuclease protection assay (RPA). These methods are designed to detect low abundance transcripts, which apply to CFTR mRNA in most cell types and tissues.

Journal of Dermatological Science (13)


http://www.sciencedirect.com/science/article/B6T87-4817HWB-1/2/8b2c2750dfb01bede7d627b022d47187

Background: Human [beta]-defensins (hBDs) belong to a group of antimicrobial peptide that are expressed in the epithelial cells. Objective: The present study investigated mRNA expression levels of the [beta]-defensins, hBD-1, -2 and -3, in human keratinocytes during differentiation in vitro. Methods: Immortalized keratinocyte cell lines, HaCaT and PHK16-0b, were used in this study; in order to stimulate differentiation, the Ca2+ concentration in the growth media was increased from 0.3 to 1.8 mM. Results: Four days after the increase, the expression levels of hBD-1 and -3 were increased in both cell lines, followed by an increase in the mRNA levels of the differentiation markers, involucrin and keratin 10. No increased expression of hBD-2 was observed. Conclusion: The results indicate that keratinocyte differentiation may stimulate hBD-1 and -3 expression in stratified squamous epithelia.


http://www.sciencedirect.com/science/article/B6T87-3RHM5V1-11/2/27f5e711dab28ab9cb8919cf6e5f5fe0

The frequency of aspartate at residue 9 (Asp-9) of HLA-C molecules was investigated among 75 Japanese patients with psoriasis vulgaris and 50 healthy controls. We developed a technique of polymerase chain reaction sequence-specific primer (PCR-SSP) amplification of genomic DNA for HLA-C alleles with a codon for Asp-9. The specificity of amplification was confirmed by direct sequencing of the amplified products and amplification from total RNA (RT-PCR). Asp-9 was positive in all individuals with Cw6 and/or Cw7, but negative in the others, indicating that Asp-9 was specific to Cw6 and Cw7 antigens in our subjects. The frequency of Asp-9 was significantly increased in the patient group (48% vs. 20%; P P < 0.0001). Asp-9 is located on a [beta] sheet of [alpha] 1 domain of HLA-C molecule and influences the peptide binding of the C pocket of the groove together with Ala-73. Both Asp-9 and Ala-73 could contribute to the disease susceptibility to psoriasis vulgaris in the immune responses.

Summary

Background: Oculocutaneous albinism (OCA) is a heterogeneous congenital disorder. Tyrosinase is a key enzyme in melanin biosynthesis, and tyrosinase gene mutations cause the OCA1 subtype. Objective: This study was intended to evaluate the frequency and details of tyrosinase gene mutations in Japanese OCA patients. Patients and methods: We examined nine non-consanguineous OCA families, sequenced the tyrosinase gene of the patients and also confirmed a splicing site mutation using exon trapping system. Results: Tyrosinase gene mutations were identified in five out of nine OCA families (55%). IVS2-10delT-a was present in 3 out of 18 alleles in three families (16%), P310insC was present in three alleles in three families (16%), and R278X was found in three alleles (16%), including those in one heterozygous and one compound homozygous patient. G97V (290 G-T) was found in 1 out of 18 alleles, and we could not find G97V in the mutation database. We have added this mutation as the 9th mutation of Japanese OCA1 patients. In 8 out of 18 alleles, no tyrosinase mutations were identified. They were presumed not to be OCA1, but other subtypes of OCA. Exon trapping system demonstrated IVS2-10delT-a mutation generated the abnormal splicing site, and inserted the codon 4 bases in mRNA level resulting in premature termination codon downstream. Conclusion: This study provided new information about OCA1 mutations, and highlights the requirement of broader detailed search to make precise diagnosis of OCA.


Background: Genetic polymorphisms of steroid 5[alpha]-reductase have been studied in androgenetic alopecia in Caucasians, but the genes encoding the two isoenzymes were not associated with male pattern baldness. Genetic polymorphisms and ethnic variations have not been studied for Asians, although it is suggested that racial difference could exist and influence clinical phenotypes. Objective: The purpose of our study is to investigate the genetic polymorphisms of steroid 5[alpha]-reductase type 1 and 2 (SRD5A1 and SRD5A2) genes in Korean population, and to study the association of these polymorphisms with the development, clinical types (female or male pattern) and therapeutic response of androgenetic alopecia. Methods: Sixty-six patients with androgenetic alopecia and controls consisted of 92 healthy men were included. Twenty-four patients were treated with finasteride for at least 6 months, and clinical responses were assessed by a simple classification. For type 1 isoenzyme, HinfI and NspI restriction fragment length polymorphisms (RFLPs) were detected using polymerase chain reaction method. For type 2 isoenzyme, RsaI RFLPs detected valine/leucine polymorphisms at codon 89, and MowI RFLPs detected alanine/threonine polymorphisms at codon 49. Results: We could not find any significant associations of the genetic polymorphisms of these two isoenzyme genes with androgenetic alopecia in Koreans (P>0.05). These polymorphisms were not associated with the clinical types of baldness or the response to finasteride (P>0.05). Conclusion: These results suggest that polymorphisms of SRD5A1 and SRD5A2 genes may not be directly associated with the development of baldness or generation of different clinical phenotypes.

Ichihashi, N. and Y. Kitajima (2000). "Loss of heterozygosity of adenomatous polyposis coli gene in cutaneous tumors as determined by using polymerase chain reaction and paraffin section
It has been suggested that an alteration in the adenomatous polyposis coli (APC) gene, which is a tumor suppressor gene, is one of the earlier events in carcinogenesis of some adenocarcinomas. We undertook this study to determine the prevalence of loss of heterozygosity (LOH) of the APC gene in several kinds of cutaneous tumors. Fifty-seven unrelated Japanese patients were examined for analysis of the APC gene. The 57 cases consisted of extramammary Paget's disease, squamous cell carcinoma (SCC), eccrine poroma and porocarcinoma, metastatic tumor of rectal adenocarcinoma and malignant melanoma. DNA was extracted from the tumor and normal portions dissected from the formalin-fixed paraffin-embedding sections and amplified with the use of the PCR. The amplified DNA was examined for LOH in the APC gene. Seven samples of 32 heterozygous persons of APC gene (three out of seven eccrine poromas, two eccrine porocarcinomas and two metastatic tumors of rectal adenocarcinoma) showed for LOH in the APC gene. None of the heterozygous samples from the extramammary Paget's disease (11), SCC (five) and melanoma (five) showed LOH. These results suggest that tumor or tumor suppressor genes, other than the APC gene, may be responsible for extramammary Paget's disease and SCC and that LOH involving APC may have some relevance to the formation and progression of eccrine tumors as in rectal tumors.


Mutations in p53, a tumor suppressor gene, are one of the most common genetic lesions of human cancers. The relationship between p53 gene mutation and ultraviolet (UV) light has been demonstrated in skin cancers of sun-exposed sites. In this study, genomic DNA from 12 skin cancers was screened for mutations in exons 5 to 9 of this gene using the polymerase chain reaction -- single strand conformation polymorphism (PCR-SSCP) analysis followed by DNA sequencing. DNA samples were obtained from 8 basal cell carcinomas (BCCs): 1 from an organoid nevus, 1 from a patient with basal cell nevus syndrome, 1 from a patient with xeroderma pigmentosum, and 1 from a recurrent and 4 from primary sporadic lesions on actinic damaged skin, and from 4 squamous cell carcinomas (SCCs): 1 from a burn scar, 1 from a patient with epidermodysplasia verruciformis, and 2 from actinic keratosis. Mutation of the p53 gene was detected in only 1 case of SCC which had arisen from actinic keratosis. The mutation occurred at codon 159 in exon S with a GCC to CCC base-pair substitution resulting in an amino acid change of alanine to proline. This mutation does not correspond to results of UV mutagenesis studies reported in the literature. Our findings imply that, although p53 gene mutation and UV exposure play an important role in the carcinogenesis of some skin cancers, they are not crucial, especially in skin cancers that develop from underlying skin disorders.

The histological diagnosis of initial mycosis fungoides (MF) and the molecular mechanisms that are responsible for its progression and transformation to the more highly malignant variants of MF remain largely unknown. Because of the rare occurrence of these tumours, the need for snap frozen skin biopsy specimens and the difficulty to obtain suitable material for karyotypic and genotypic analysis, specific cytogenetic and molecular lesions have not yet been identified. In particular the role of known oncogenes and tumour suppressor genes, including the p53 gene, in the pathogenesis and clinical progression of MF has not been extensively investigated. The present study was carried out using the polymerase chain reaction (PCR) technique combined with temperature gradient gel electrophoresis (TGGE) to detect mutations of the p53 gene in 58 patients with MF. TGGE analysis was also used in combination with clonality analysis by means of T-cell receptor [gamma] (TCRG) gene rearrangement studies to distinguish parapsoriasis en plaque and initial MF from patch/plaque stage MF. More than 83% of the diagnoses of initial MF could be confirmed using PCR-TGGE analysis. However, although the sensitive TGGE analysis was used for all exons, p53 gene polymorphisms were found in 4 and p53 gene mutation in only 1 of 58 biopsy specimens. It appears unlikely that p53 gene mutations play a role in either the pathogenesis of parapsoriasis and initial MF or their progression to advanced stages of MF. However, TCRG gene rearrangement studies by means of TCR-TGGE analysis may be useful for distinguishing histologically discordant cases of initial MF.


http://www.sciencedirect.com/science/article/B6T87-408KCH0-6/2/2944f2cde732fa8c830cd8b8006a9

A combination of haplotype analysis and direct sequencing were conducted on Japanese Muir-Torre syndrome kindred. In the kindred, two females revealed a hereditary non-polyposis colon cancer (HNPCC) phenotype and one male had a sebaceous tumor in addition to a HNPCC phenotype. Haplotype analysis and direct sequencing failed to show involvement of the known mismatch repair genes, with the exception of MSH5, in this kindred. Analysis of large fragments (from 3.9 to 6.2 kb) covering the entire 25 kb MSH5 gene in the proband revealed the absence of gross changes in the promoter region and exons. The direct sequencing of the promoter region and all 25 exons failed to demonstrate any mutations in the coding regions except for a CA repeat polymorphism in intron 3 and a C/A polymorphism in intron 15. Taken together present results indicate that a novel and yet unknown mismatch repair gene is likely involved in the HNPCC in this kindred.


http://www.sciencedirect.com/science/article/B6T87-46TG2X9-3/2/53e544f70b50c01166e43e502f4367

Skin sulfhydryl oxidase (SOx) is an enzyme that catalyzes disulfide (S-S) cross-linking through the oxidation of sulfhydryl compounds in the skin. In this study, using the enzyme purified from rat seminal vesicle, we obtained peptide sequences for SOx by mass spectrometry. We then searched for SOx nucleotides corresponding highly to the rat peptide sequences by assembling murine-expressed sequence tags (ESTs) from the GeneBank database. The assembled mouse SOx cDNA has an open reading frame of 1704-bp nucleotides, translating into a size of 568 amino acids. The calculated molecular mass of the mouse SOx protein is 65 kDa. This mouse
The sequence can be amplified from total RNAs of various mouse tissue samples by reverse transcription polymerase chain reaction, especially highly amplified from those of the seminal vesicles and epidermis. The cDNA fragment was subsequently cloned into the mammalian expression vector (pTARGET-MSSOx), allowing us to express mouse recombinant SOx protein in cultured cells. When pTARGET-MSSOx was transfected, Western blot analysis using anti-SOx antiserum could detect a 65 kDa-band of recombinant SOx in both samples from the whole cell extract and the medium after the harvest of the HEK cells. In immunohistochemical analysis, the Pt-K2 cells, following the introduction of pTARGET-MSSOx, seemed to generate a SOx protein reactive to anti-SOx antiserum in the cells. Moreover, the indirect staining of the S-S bonds using N-(7-dimethylamino-4-methyl coumarinyl) maleimide (DACM), following the addition of N-ethylmaleimide and dithiothreitol, showed that the formation of S-S bridges almost matched the localization of SOx expression in the Pt-K2 cells after the transfection. In essence, we cloned skin SOx cDNA and characterized it as one of the S-S cross-linking enzymes. The SOx clone from mouse epidermis seems to be useful for investigating the potential function of the enzyme in the epidermis, especially for understanding the physiological role of SOx in the differentiation of keratinocytes.


http://www.sciencedirect.com/science/article/B6T87-4DBKGX9-3/2/f88341acbe6ede5eb41785f994682e

SummaryBackground:Mucosal high-risk human papillomaviruses (HPVs), such as type 16, are detectable in oral cancers, especially of the oropharynx and tonsils, and there is evidence that they play a pathogenetic role in some cases. However, information is limited about their significance for cancers of the vermilion of the lip.Objective:To determine the detection rate, types and localization of HPVs in squamous cell carcinomas (SCCs) of the lip.Methods:Nested PCR for cutaneous HPVs, including epidermodysplasia verruciformis-related HPV (EV-HPV), and single PCR for mucosal HPVs, were conducted for a total of 27 SCCs and normal samples from 30 individuals. Tyramide-based in situ hybridization (ISH) was also applied.Results:Various types of HPVs were detected, particularly from normal individuals. Among the kinds of the HPV types detected in this study, half were found by PCR using a primer pair, which we newly designed. The prevalence of HPV was 5 out of 27 SCCs (ca. 18%) and 10 out of 30 normal individuals (ca. 33%). They were the entire cutaneous-group except for two, from one SCC and one normal individual.Conclusion:On the surface of the normal lip various types of mainly cutaneous-group HPVs may be present, but there does not appear to be any obvious association with SCCs developing in this site.


http://www.sciencedirect.com/science/article/B6T87-3SBW2FT-4/2/407b7f9c7607a10c7b9fd2aa084d4d4fc8

We describe here a newly established cell line from an eccrine carcinoma which produced an abundant amount of granulocyte colony-stimulating factor (G-CSF). An eccrine carcinoma of the scalp of a 69 year-old-Japanese female had metastasized to the pleura. Clinically, she had marked neutrophilia (up to 60000/mm3), and a high level of G-CSF (38.7 x 103 pg/ml) was detected in the pleural effusion, as determined by enzyme-linked immunosorbent assay (ELISA).
We established a cell line in vitro and maintained the cells in culture for 30 months in 90 subcultures. We investigated whether these tumor cells were able to produce G-CSF in culture and found that they were. We also found that the amount of G-CSF produced paralleled the rise in cell number (26.5 x 103 pg/ml at confluence). When culture media were administered to rabbits (25 ml/rabbit), the amount of circulating neutrophils increased until the number was equal to or greater than that resulting from injection of recombinant human G-CSF (rhG-CSF)(75 [mu]g). This effect persisted for 7 days. When tumors were induced in SCID and nude mice by injecting cultured cells (1 x 107 cells/mouse), the number of circulating neutrophils also correlated well with tumor size in these mice (200000/ mm3, 3 cm tumor). After tumor removal, the neutrophil number returned to normal within 30 days. G-CSFmRNA in cultured, cells was detected by RT-PCR. Based on these results, it was confirmed that the marked neutrophilia observed in the patient was caused by the tumor-generated G-CSF. This is the first G-CSF-producing cell line developed from a cancer of the skin.


http://www.sciencedirect.com/science/article/B6T87-4FDJRVR-1/2/35af1d6b8250e4fa772494780a81d427


http://www.sciencedirect.com/science/article/B6T87-3VTHPY3-7/2/a8dabdb6f57b85657a7f6d6388bccbc2

Keratin intermediate filaments are expressed in specific type I/type II pairs in the stage of differentiation of keratinocytes. The mutations in the keratin genes expressed in the epidermis are etiologically responsible for several epidermal genetic skin diseases, such as epidermolysis bullosa simplex, epidermolytic hyperkeratosis (EHK), ichthyosis bullosa of Siemens, palmoplantar keratoderma, pachyonychia congenita and white sponge nevus. The mutations of keratins 1/10 which are expressed in spinous and granular layers are confirmed to cause EHK. There are several trials to correlate between the clinical phenotypes and sites of mutations of the keratin genes. One of these is that EHK is divided into two groups: the palms and soles involvement (PS) group and the non-palms and soles (NPS) group. So far the PS group had the mutations in the keratin 1 and the NPS group in keratin 10. Most of the mutations of the NPS group were reported in the beginning of the 1A rod domain and over 2/3 of the mutations in the 1A rod domain were the base pair substitution of arginine. Here we find two different mutations in two unrelated Korean kindreds classified as NPS group--R156C and R156H--in the 1A rod domain of keratin 10. Our results are compatible with the above classification and suggest that the arginine in the beginning of the 1A rod domain is the hot spot for the mutation of the keratin 10 gene.
Increased production of reactive oxygen species (ROS) has been suggested as a cause of diabetic complications. Uncoupling proteins (UCPs) have been ascribed a role in reducing the formation of ROS, and genetic variation in genes encoding for UCPs could thus be putative candidate genes for diabetic nephropathy. To test this hypothesis we searched for association between the A->G (-3862) variant in UCP1, the insertion/deletion (I/D) polymorphism in exon 8 in UCP2, and the C->T (-55) polymorphism in UCP3 and diabetic nephropathy in 218 diabetic patients with normal urinary albumin excretion rate (AER), 216 with micro- or macroalbuminuria, and in 106 control subjects without a family history of diabetes. We did not find any association between the different polymorphisms and diabetic nephropathy, nor did we observe any difference in AER among carriers of different UCP1-3 genotypes. We could, however, confirm the reported association between BMI and the UCP3 -55 C->T polymorphism; patients carrying the T allele had higher BMI than patients homozygous for the C allele (26.4+/-.2 vs. 25.3+/-.4.3 kg/m2; P=.01). We conclude that studied polymorphisms in the UCP1-3 genes do not play a major role in the development of micro- or macroalbuminuria in Scandinavian diabetic patients.

Advanced glycation end product (AGE) engagement of a cell surface receptor for AGE (RAGE) has been implicated in the development of diabetic complications. In this study, we determined the RAGE mRNA levels in monocytes from type 1 diabetic patients and analyzed their relationship with diabetic vascular complications. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that the monocyte expression of RAGE mRNA was significantly lower in patients with retinopathy than in those without retinopathy and was also significantly down-regulated in patients with nephropathy in comparison with those without nephropathy. Experiments with monocyte-enriched cultures revealed that RAGE mRNA and protein levels were down-regulated by the exposure to glyceraldehyde-derived AGE--the recently identified high-affinity RAGE ligand. Accordingly, we then assayed for the serum levels of glyceraldehyde-derived AGE as well as those of carboxymethyllysine (CML)--the known RAGE ligand and related them to the monocyte levels of RAGE mRNA. This screen revealed a negative correlation between the two parameters. The results thus suggest that the decrease in monocyte RAGE expression can be at least partly accounted for by the ligand engagement and may be a factor contributing to the development of diabetic vascular complications.
Galactose-fed dogs develop retinal capillary changes similar to diabetic retinopathy with pericyte degeneration as the initial lesion. This is followed by the formation of microaneurysms, hemorrhages, and some areas of acellularity. To investigate the mechanisms for selective pericyte degeneration, retinal capillary pericytes and endothelial cells isolated from beagle dog retina were cultured for 2 weeks in Dulbecco's modified Eagle's medium (DMEM) containing 50 mM galactose. Apoptosis was detected in pericytes but not endothelial cells by in situ terminal deoxynucleotidyl transferase (TdT)-mediated biotin-dUTP nick end labelling (TUNEL) staining and the DNA fragmentation assay on agarose gel electrophoresis. This apoptosis was prevented by the addition of the aldose reductase inhibitor AL 1576 to the culture medium containing galactose. Apoptosis was not observed when pericytes were similarly cultured in control DMEM medium. These data support the premise that the selective degeneration of retinal capillary pericytes observed in galactose-fed dogs is linked to increased aldose reductase activity in these cells.

Journal of Experimental Marine Biology and Ecology (7)


http://www.sciencedirect.com/science/article/B6T8F-3W2515R-1/2/9ed4f3bc90f62bda0889203d8785ebaa

Anonymous nuclear DNA polymorphisms were developed for the marine mussel Mytilus by cloning random segments of nuclear DNA, sequencing the ends, constructing primers to amplify the cloned segments, and restricting the PCR product. The technique of PCR RFLP analysis was applied, for three cloned segments, using genomic DNA preparations from the three closely related mussel species, Mytilus edulis L., Mytilus galloprovincialis (Lamarck) and Mytilus trossulus (Gould) sampled from European populations. M. trossulus was found to differ at the nucleotide level from the other two species which were closely similar. This result contrasts with that obtained for mtDNA where M. edulis and M. trossulus have higher resemblance, and for allozymes where genetic distance values between the species are similar. The contrasting results for mtDNA and nuclear polymorphisms can be attributed to extensive mtDNA gene flow between M. edulis and M. trossulus.


http://www.sciencedirect.com/science/article/B6T8F-4950C78-1/2/65233e8030210dc362121fa4db215146

Closely related species, Penaeus merguiensis and Penaeus silasi from Thai waters, were genetically examined using variation observed in 558 base pairs (bp) of sequence from cytochrome oxidase subunit I (COI) gene of mtDNA. The sequence divergences of COI between P. merguiensis and other Penaeus species were 5.76-6.15% (P. silasi), 13.17-13.97% (Penaeus indicus), 16.43% (Penaeus vannamei), 16.63% (Penaeus monodon), and 18.37% (Penaeus
From the alignment reported that there were four clades on phylogenetic tree, the distinction of the two monophyletic clades was referred as P. merguiensis, one monophyletic clade within P. silasi and P. indicus. These results point toward the possibility of P. merguiensis being a complex of two cryptic species or a single species with strong phylogeographic subdivision.


In the fall, freeze tolerant intertidal invertebrates usually produce ice-nucleating proteins that are secreted into the hemolymph. These proteins help protect against freeze damage by insuring that ice formation is limited to extracellular spaces. Geukensia demissa, a freeze tolerant, salt marsh bivalve mollusc was examined for the presence of ice nucleating proteins. The ice-nucleating temperature (INT) of the hemolymph was not significantly different from artificial seawater of the same salinity indicating the lack of an ice nucleating protein in the hemolymph. The palial fluid did have an elevated INT, indicating the presence of an ice nucleator. The INT of the palial fluid was significantly reduced by boiling and filtration through a 0.45-[mu]m filter. High INT was also observed in the seawater associated with the bivalves, and was demonstrated in water samples collected from salt marshes but not sand and pebble beaches. Moreover, the INT of water samples collected from a salt marsh decreased in the summer. All of these data suggest that the ice-nucleating agents in the hemolymph and the seawater are ice-nucleating bacteria. One species of ice-nucleating bacteria, Pseudomonas fulva was isolated from the gills of Geukensia. These bacteria could perform the same function as hemolymph ice-nucleating proteins by limiting ice formation to extracellular compartments.


Predator-prey interactions play an influential role in determining the demographics of a population or species. In the Northwest Atlantic, Atlantic cod, Gadus morhua, once the basis of a lucrative commercial fishery, have not recovered despite regulations imposed on the fishery to reduce harvest rates. One possible reason for the lack of recovery is that high predation pressure on juvenile and larval stages, particularly from species such as herring and mackerel, may regulate the abundance of cod. However, traditional methods used to identify larval cod and haddock often fail when applied to partially digested remains. Here, we described a DNA-based assay to identify the presence of digested cod remains from the stomachs of predatory fish species. After development, the assay was tested on two sets of field samples. Larval and juvenile cod were successfully detected in both tests.

In its habitat, Chasmagnathus granulata is exposed to many different environmental challenges according to the season of the year. For this reason, the investigation of the participation of the gluconeogenic pathway in the acclimation to hypo- and hyperosmotic conditions in summer and winter was considered interesting. By comparing the gluconeogenesis capacity, phosphoenolpyruvate carboxykinase (PEPCK) activity, and mRNA PEPCK gene expression values obtained in control crabs during summer and winter, we found two opposite tendencies: a decrease in winter and an increase in summer. The present results show that, in the C. granulata jaw muscle, PEPCK activity is divided almost equally between the cytosol and mitochondria in winter crabs. This distribution is different from the one found in muscle from summer crabs, in which most of the PEPCK activity (85%) takes place in the mitochondria. The data reported here show that the natural light/dark cycle typical for winter regulated the proportion of PEPCK activity in the cytosolic and mitochondrial fractions at the transcriptional level, with a marked decrease in the mitochondrial PEPCK activity and, as a result, in the gluconeogenic capability. The gluconeogenic activity decreased 48% after 24 h of hyperosmotic stress in summer. Furthermore, this treatment reverted the proportion of PEPCK activity in cytosolic and mitochondrial fractions: it decreased in the mitochondrial fraction and increased in the cytosolic one. However, at 72 h of hyperosmotic shock, the incorporation of label from alanine into glucose increased 45% as compared to a 24-h group. Hence, it is possible that the increase in gluconeogenic capacity after 72 h of hyperosmotic stress is due to the enhanced PEPCK gene expression at 24 h of osmotic stress. The present study shows that the increases in the incorporation of 14C-alanine into glucose and in the mitochondrial and cytosolic activities in the jaw muscle of C. granulata after 24 h of acclimation to a dilute media in summer did not require an increase in PEPCK gene expression. During hypooosmotic shock in winter, the gluconeogenenic capacity and the PEPCK activity are still remarkably low, and the PEPCK gene expression remains undetectable. The muscle gluconeogenesis seems to be one of the pathways implicated in the metabolic adjustment during hypo- and hyperosmotic shock in C. granulata. On the other hand, the present study highlights the importance of seasonal environmental differences in determining the development of metabolic patterns.


The deleterious effects of temperature-induced coral bleaching, a process by which corals lose their endosymbiotic algae (zooxanthellae; genus Symbiodinium) primarily at temperatures above mean yearly maximums, has not been well described for alcyonacean soft corals (Coelenterata, Octocorallia). The study of Symbiodinium cells lost from Sarcophyton ehrenbergii, Sinularia sp., and Xenia sp., which have not been compared in bleaching studies, indicate that the soft coral S. ehrenbergii released the greatest number of symbiont cells, however, it was less susceptible to heat stress surviving temperatures of 34 [deg]C for >39 h. Sinularia sp. showed intermediate levels of bleaching tolerance to elevated temperatures, surviving prolonged exposures at 32 [deg]C, but dying within 24 h at 34 [deg]C. Xenia sp., however, was the most vulnerable to high heat stress maximally releasing Symbiodinium at temperatures [less-than or equal to]30 [deg]C. This evidence indicates that Xenia sp. is even more susceptible to elevated temperatures than Acropora spp., previously reported to be the most vulnerable coral species to elevated temperature-induced bleaching. Molecular analysis showed that the more resistant soft coral species (S. ehrenbergii) had the same type of Symbiodinium (clade C) as less resistant soft corals (Xenia sp.). In comparison to scleractinian corals collected from the same region that show
similar bleaching resistance to high temperatures (e.g. Porities solida—more robust; Favites complanata—moderate resistance; Acropora hyacinthus—less robust), all scleractinian corals were symbiotic with Symbiodinium from clade C. A. hyacinthus, however, was found to possess multiple symbionts (clades B and C), and this represents a first report of Clade B in any Acropora species.


http://www.sciencedirect.com/science/article/B6T8F-3YGDCSP-8/2/0d8701129cd355e5932b01f996e10d50

We report the nucleotide and deduced amino acid sequences of Pem-CMG peptide, a member of crustacean CHH/MIH/GIH peptide family, in black tiger prawn (Penaeus monodon). The 5' and 3' fragments of Pem-CMG cDNA were cloned by the method of rapid amplification of cDNA ends (RACE). The two fragments constitute a combined cDNA length of 593 bp with a 77 bp overlapping region. Sequence analysis reveals the presence of a 384 bp open reading frame which was subsequently cloned. The open reading frame encodes a precursor peptide that is comprised of 128 amino acids, with a putative processing site, KR. The mature peptide consists of 74 amino acid residues, the sequence of which is significantly homologous to those of the CHH/MIH/GIH family known from other crustaceans. Analysis of a genomic fragment of Pem-CMG reveals a single intron of 314 bp interrupting the coding sequence for the mature peptide. The presence of only one intron in Pem-CMG gene suggests that this gene is structurally different from the previously reported MIH gene of Charybdis feriatus and CHH-like gene of Metapenaeus ensis which possess two introns in their coding sequences.

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cis (1S,2R) indandiol or trans (1R,2R) indandiol are both potential precursors to (-)-cis (1S,2R)-1-aminoindan-2-ol, a key chiral synthon for Crixivan(R) (Indinavir), a leading HIV protease inhibitor. Enrichment and isolation studies yielded two Rhodococcus sp. strain B 264-1 (MB 5655) and strain I-24 (MA 7205) capable of biotransforming indene to cis (1S,2R) indandiol and trans (1R,2R) indandiol respectively. Isolate MB 5655 was found to have a toluene dioxygenase, while isolate MA 7205 was found to harbor both toluene and naphthalene dioxygenases as well as a naphthalene monoxygenase. When scaled up in a 14-l bioreactor, MB 5655 produced up to 2.0 g/l of cis (1S,2R) indandiol with an enantiomeric excess greater than 99%. MA 7205 cultivated under similar conditions produced up to 1.4 g/l of trans (1R,2R) indandiol with an enantiomeric excess greater than 98%. Process development studies yielded titers greater that 4.0 g/l of cis indandiol for MB 5655. Due to their resistance to indene toxicity and easy cultivation in
bioreactors, both Rhodococcus sp. strains appeared as good candidates for future strain engineering and process development work.


http://www.sciencedirect.com/science/article/B6T8G-4865H3Y-8R/2/f12392d089e0d349ff9da80d0a6ba66a

Two cDNAs, prxCa and prxEa, encoding peroxidase isozymes of Arabidopsis thaliana, ARP Ca and ARP Ea, respectively, were isolated by polymerase chain reaction using total RNA from stem and primers designed from genomic DNAs. The coding regions of the prxCa and prxEa cDNAs were identical to the putative exon regions in the genomic DNAs (intapruk, C. et al., Gene, 98, 237-241, 1991) and contained 1092 and 1078 bp, respectively. The deduced amino acid sequences of ARP Ca and ARP Ea suggested the presence of leader peptides of 31 and 29 amino acid residues at the N-termini, and 308 and 307 amino acid residues in mature proteins, respectively. The amino acid sequence of ARP was compared with those of other plant peroxidases: ARP showed highly conserved alignment with the horseradish peroxidase (HRP) family (group I), with 64 to 91% homology except for HRP n; lower similarity to rice, tobacco, cucumber, turnip, wheat and peanut peroxidases (group II) with 40 to 51% homology; and a very low level of homology of about 37% for potato and tomato peroxidases. The prxCa and prxEa were close to HRP neutral and basic isozyme genes, respectively, but the estimated isoelectric points of ARP Ca and ARP Ea showed values of 8.05 and 6.34, respectively. By Northern blot hybridization, the prxCa gene was shown to be expressed non-specific expression in many organs of A. thaliana, while the level of transcripts of the prxEa gene was found to be abundant in root but very low in leaf and stem.


http://www.sciencedirect.com/science/article/B6T8G-3RXXYBF-F/2/61e72c887f2cb34b633d625faa8181e1

The gene encoding the single cellulose-binding domain II (CBD II) of Clostridium stercorarium xylanase A was fused to theeglIV gene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The fusion protein (EGIV + CBDII) expressed in Escherichia coli can be readily purified from the cell-free extract of E. coli in a single step using the affinity of CBD to cellulose. The purified enzyme was cleaved into two moieties, i.e. the catalytic domain and CBD, at a specific site in the linker region by partial digestion with trypsin at 4[deg]C. This result indicates that this CBD belonging to family VI of CBD families can be used as an affinity tag for purification of the recombinant protein.


http://www.sciencedirect.com/science/article/B6T8G-3S6MP0K-
The chiA gene encoding a chitinase of 60-kDa has been cloned from Enterobacter sp. G-1 by PCR using synthetic oligonucleotides corresponding to the amino acid sequences of the purified enzyme and subsequently genomic library screening was performed. The products of the positive clones were found to degrade water-insoluble chitin. The primary structure of the chiA gene consisted of 1,686-bp encoding 562 amino acid residues. Comparison of the deduced amino acid sequence of the cloned chitinase gene product (chiA) with other chitinases revealed a high homology (95.7% identity) with chitinase A from Serratia marcescens QMB1466. The coding region of the chiA gene for higher expression in Escherichia coli was identified using deletion and sequence analysis. The expression of the chiA gene in Enterobacter sp. G-1 was controlled by presence of chitin, as determined by Northern blotting hybridization analysis. We found that the expression of the chiA gene in E. coli was controlled by an inverted repeat sequence located in the upstream region from a promoter region.


http://www.sciencedirect.com/science/article/B6W8T-44423W2-8C/2/21da48e0129a76674995a5ed0c207631

The Barrett's multistage process is characterized histopathologically by progression from Barrett's intestinal metaplasia to Barrett's esophagus with dysplasia and ultimately adenocarcinoma. Understanding the cellular and molecular events in this multistage process may contribute to improved diagnosis and treatment. Ornithine decarboxylase (ODC) is the first enzyme in the biosynthesis of polyamines. Elevated ODC activity has been found to be associated with progression during Barrett's esophagus, but the regulation of ODC gene expression in the development of Barrett's-associated adenocarcinoma has not been reported. The aim of this study was to assess the prevalence and timing of ODC mRNA expression in the Barrett's metaplasia-dysplasia-carcinoma sequence. ODC mRNA expression levels, relative to the stably expressed internal reference gene [beta]-actin, were measured using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method (ABI 7700 Sequence Detector System) in 104 specimens from 19 patients with Barrett's esophagus without carcinoma and 22 patients with Barrett's-associated adenocarcinoma. The median ODC mRNA expression levels were significantly increased in Barrett's esophagus tissues compared to matched normal tissues in patients without adenocarcinoma of the esophagus (P = 0.002; Wilcoxon test). A significant progressive increase in ODC mRNA expression was detectable through the stages of the metaplasia-dysplasia-carcinoma sequence in patients with Barrett's-associated adenocarcinoma (r = 0.719; P <=0.001; Spearman's rho test). These findings show that upregulation of ODC mRNA expression is an early event in the development and progression of Barrett's-associated adenocarcinoma of the esophagus, and they suggest that high ODC mRNA expression levels may be a clinically useful biomarker for the detection of occult adenocarcinoma.

The Barrett's multistage process is characterized histopathologically by progression from Barrett's intestinal metaplasia to Barrett's esophagus with dysplasia and ultimately adenocarcinoma. Understanding of the molecular alterations in this multistage process may contribute to improved diagnosis and treatment. Retinoid X receptors (RXR) play an important role in regulating the morphogenesis, development, growth, and differentiation of cells. Alterations in RXR expression have been observed in a variety of solid tumors; however, the role in Barrett's esophagus disease has yet to be determined. The aim of this study was to assess the prevalence and timing of RXR messenger RNA expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence and to investigate its role in the development and progression of this disease. We analyzed the mRNA expression of all three RXR subtypes (RXR-alpha, RXR-beta, and RXR-gamma) by using a quantitative real-time reverse transcription-polymerase chain reaction method in 108 specimens from 19 patients with Barrett's esophagus without carcinoma (BE group), 20 patients with Barrett's-associated adenocarcinoma (EA group), and a control group of 10 patients without evidence of gastroesophageal reflux disease (CG). RXR-[alpha] mRNA expression was significantly decreased (P = 0.01; Wilcoxon test) and adenocarcinoma tissues (P = 0.018, Mann-Whitney test). RXR-[alpha] and RXR-[beta] mRNA expression was significantly associated in normal squamous esophagus tissues (r² = 0.49; P = 0.001). There were significant differences in RXR-[alpha] (P = 0.011) and RXR-[beta] (P = 0.005) mRNA expression in histopathologically normal squamous esophagus tissues in patients with cancer and the control group without evidence of gastroesophageal reflux disease. These findings suggest that alterations in the mRNA expression of all three RXR subtypes are frequent events in the development and progression of Barrett's esophagus and associated adenocarcinoma, that RXR mRNA expression levels may be useful biomarkers for this disease, and that a widespread "field-effect" is present in the normal esophagus of patients with esophageal adenocarcinoma.
group, the median GSTPI mRNA expression was highest in normal squamous esophagus epithelium, intermediate in Barrett's esophagus, and lowest in adenocarcinoma tissues (P = 0.001) or the EA group (P = 0.023). GSTPI expression levels in adenocarcinoma tissues were decreased compared to matching normal esophagus tissues from the patients with adenocarcinoma (P = 0.011). Furthermore, GSTPI mRNA expression values were significantly different between metaplastic, dysplastic, and adenocarcinoma tissues (P = 0.026). GSTPI expression levels were also significantly lower in histologically normal squamous esophagus tissues from patients with cancer (EA group) compared to both normal esophagus tissues from patients without cancer (BE group; P = 0.007) and normal esophagus tissues from the control group with no esophageal abnormality (P = 0.002). GSTPI protein expression was generally highest in the basal layer of normal squamous esophagus epithelium and lowest in adenocarcinoma cells, with Barrett's cells showing intermediate staining intensity. Our results show that downregulation of GSTPI expression is an early event in the development of Barrett's esophagus and esophageal adenocarcinoma. Loss of GSTPI expression may have an important role in the development and progression of this disease. (2002;6:359-367.)


http://www.sciencedirect.com/science/article/B6W8T-4D9VBRY-2/2/bf3c80631fd02e3b6908430376508d8d

Gastrointestinal motility is strongly inhibited during peritonitis or sepsis and proinflammatory cytokines released into mesenteric lymph during an acute gastrointestinal insult mediate systemic responses. We investigated whether mesenteric lymph collected during peritonitis or sepsis inhibits gastric motility and gastric emptying. Mesenteric lymph was collected for 12 hours from three experimental groups: vehicle (saline, 1 ml, intraperitoneally [ip], control lymph), peritonitis (0.5% acetic acid, 1ml, ip, peritonitis lymph), and sepsis (lipopolysaccharide [LPS], 5 mg/kg, 1 ml, ip, sepsis lymph). Gastric motility and gastric emptying were measured in recipient rats in response to lymph injections into the jugular vein. Quantitative polymerase chain reaction (PCR) for tumor necrosis factor [alpha] (TNF[alpha]) gene expression in the jejunum and in lymph cells were measured during sepsis. Mesenteric lymph flow significantly increased during peritonitis or sepsis (lymph flow [ml] per 60 minutes; control 2.45 [plus-or-minus sign] 0.04; peritonitis 2.67 [plus-or-minus sign] 0.07; sepsis 3.25 [plus-or-minus sign] 0.1, p 2O, 1.89 [plus-or-minus sign] 1.31 minutes; peritonitis lymph: -0.56 [plus-or-minus sign] 0.06 cm H2O, 9.9 [plus-or-minus sign] 0.9 minutes; sepsis lymph: -0.51 [plus-or-minus sign] 0.05 cm H2O, 6.9 [plus-or-minus sign] 0.6 minutes; p p < 0.001 vs. basal; 12 hours: 24.7 [plus-or-minus sign] 16.8, not significant [NS]; 24 hours: 7.0 [plus-or-minus sign] 3.4, NS). In conclusion, mediators in mesenteric lymph, possibly cytokines, may be responsible for the inhibition of gastric motility during peritonitis or sepsis. Because the composition of mesenteric lymph probably reflects the interstitial fluid of the gut wall, monitoring visceral lymph might be an extremely beneficial tool to determine mediators released during impaired gut wall function.


http://www.sciencedirect.com/science/article/B6W8T-4DYV0NT-J/2/6168ee832eaf88fdfad25a84924abe1a

Cyclooxygenase (Cox-2) is implicated in the pathogenesis of many cancers including esophageal adenocarcinoma (EAC), whereas the role of the isoform Cox-1 in carcinogenesis is not well
understood. To further elucidate the role of these factors in the development of EAC, we measured the gene expressions (mRNA levels) of Cox-2 and Cox-1 by real-time quantitative polymerase chain reaction (QRT-PCR) in tissues from normal esophagus with and without erosive gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), dysplasia, adenocarcinoma, and in healthy gastric antrum. All tissues were purified by laser capture microdissection from endoscopic or surgical resection specimens. Median Cox-2 gene expression did not differ significantly among the esophageal control groups but was elevated 5-fold in BE, 8-fold in dysplasia and 16-fold in EAC compared to normal esophageal controls with no erosive GERD. Erosive GERD tissue had slightly higher median Cox-2 expression but Cox-2 expression in normal antrum was much higher than that in a normal esophagus, close to that of dysplasia. In contrast to that of Cox-2, Cox-1 expression was significantly decreased in all neoplastic tissues compared to normal controls. Cox-1 and Cox-2 expression varied over a wide range in the neoplastic tissues but over a relatively narrow range in the esophageal normal tissues. The occurrence of substantial alterations in Cox-1 and Cox-2 expression at the BE stage indicates that these are early events in the development of EAC. These results confirm the important role of Cox-2 amplification in the pathogenesis of esophageal adenocarcinoma, but the unexpected down-regulation of Cox-1 raises questions about its role in carcinogenesis.


http://www.sciencedirect.com/science/article/B6W8T-4442JP3-FS/2/c796073fd269f8a183e99a8cb5aeb0c8

Barrett's esophagus is a multistage polyclonal disease that is associated with the development of adenocarcinoma of the esophagus and esophagogastric junction. Telomerase activation is associated with cellular immortality and carcinogenesis, and increased expression of the telomerase reverse transcriptase catalytic subunit (hTERT) has been used for the early detection of malignant diseases. To identify biomarkers associated with each stage of the Barrett's process, relative mRNA expression levels of hTERT were measured using a quantitative reverse transcription–polymerase chain reaction method (ABI 7700 Sequence Detector (TaqMan system) in Barrett's intestinal metaplasia (n = 14), Barrett's dysplasia (n = 10), Barrett's adenocarcinoma (n = 14), and matching normal squamous esophageal tissues (n = 32). hTERT expression was significantly increased at all stages of Barrett's esophagus, including the intestinal metaplasia stage, compared to normal tissues from patients without cancer (intestinal metaplasia vs. normal esophagus, P = 0.001; adenocarcinoma, P = 0.007; all Mann-Whitney U test). hTERT expression levels were significantly higher in adenocarcinoma tissues than in intestinal metaplasia tissues (P = 0.003), and were higher in dysplasia compared with intestinal metaplasia tissues (P = 0.056). hTERT levels were also significantly higher in histologically normal squamous esophageal tissues from cancer patients than in normal esophagus tissues from patients with no cancer (P = 0.013). Very high expression levels ([hTERT x 100; [beta]-actin] > 20) were found only in patients with cancer. These findings suggest that telomerase activation is an important early event in the development of Barrett's esophagus and esophageal adenocarcinoma, that very high telomerase levels may be a clinically useful biomarker for the detection of occult adenocarcinoma, and that a widespread cancer "field" effect is present in the esophagus of patients with Barrett's cancer.


http://www.sciencedirect.com/science/article/B6W8T-49V9HDY-
Lymph node involvement is an important prognostic factor in intrahepatic cholangiocarcinoma. Besides the nodes in the hepatoduodenal ligament, recent studies have suggested that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature can be affected when the primary tumor is located in the left hepatic lobe. However, the distribution of metastatic nodes has not been well described in this disease. Thirteen patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study. Lymphatic mapping was performed by means of both histologic examination and reverse transcriptase-polymerase chain reaction assays. Nodal involvement around the cardiac portion of the stomach or along the lesser gastric curvature (left pathway) was found in 7 (54%) of 13 patients by histologic examination or reverse transcriptase-polymerase chain reaction, whereas positive nodes in the hepatoduodenal ligament (right pathway) were found in 6 (46%) of 13 patients. Two patients (15%) had positive nodes only in the left pathway. Therefore, for a more accurate clinical staging of intrahepatic cholangiocarcinoma in the hepatic left lobe, lymph nodes around the cardiac portion of the stomach and along the lesser gastric curvature should be examined in addition to nodes in the hepatoduodenal ligament.


http://www.sciencedirect.com/science/article/B6W8T-45TY7F9-12/2/ba96303a02810beedb2dc9f4ccd44fbb

Increasing evidence supports the existence of regulatory T cells that may inhibit the allogeneic immune response after transplantation by secreting regulatory cytokines. To determine whether rat liver tolerance is associated with intrahepatic regulatory T cells secreting a characteristic cytokine profile, we analyzed the cytokine production of freshly isolated intragraft CD4+ T cells at different times postoperatively by semiquantitative reverse transcription-polymerase chain reaction and by enzyme-linked immunosorbent assay before and after in vitro stimulation. Orthotopic arterialized liver transplantation was performed in two allogeneic rat strain combinations, one with fatal acute rejection (DA-to-LEW) and one with long-lasting tolerance (LEW-to-DA) without immunosuppression despite a complete major histocompatibility complex mismatch (spontaneous liver tolerance). Liver allografts of both groups showed continuously increasing cellular infiltration between day 3 and day 7 after transplantation. In this inflammatory situation, very low levels of interleukin-13 were detectable directly after cell isolation, as well as after in vitro stimulation. However, after 30 days, intrahepatic CD4+ T cells in the tolerance group were then able to express elevated messenger RNA levels of the anti-inflammatory cytokine interleukin-13 in response to stimulation. This result indicates the presence of an intragraft Th2-like CD4+ T cell population, which may have a regulatory function in the induction of liver tolerance. (2002;6:455-463.)

Background/Aims: Recently, several cells found within the liver have been reported to derive from bone marrow (BM). This study sought to examine the commitment of BM cells to hepatic stellate cell (HSC) lineage in mouse liver.

Methods: We transplanted BM cells from green fluorescent protein (GFP) transgenic mice into age-matched C57BL/J mice. Hepatic nonparenchymal cells were isolated from the livers of BM-transplanted mice using density gradient centrifugation with Nycodenz. The expression of lineage markers by the isolated cells was evaluated by RT-PCR and immunostaining. We then examined the histology of liver tissues obtained from BM-transplanted mice with and without carbon tetrachloride-induced injury.

Results: GFP-expressing cells with intracytoplasmic lipid droplets comprised 33.4 ± 2.3% of the cells isolated by density gradient centrifugation. These cells expressed the HSC lineage markers, such as desmin and glial fibrillary acidic protein (GFAP), by both RT-PCR and immunostaining. During a 7-day culture, GFP-positive cells began to express [alpha]-smooth muscle actin, a marker of activated HSC. In the liver of BM-transplanted mice, GFP-positive nonparenchymal cells expressed GFAP and extended their process around hepatocytes. Upon liver injury, these cells also co-expressed desmin and [alpha]-smooth muscle actin.

Conclusions: Nonparenchymal cells, derived from transplanted BM, acquired HSC characteristics in both quiescent and activated states.


Background/Aims: Peroxisome proliferator-activated receptor-[gamma], which is involved in the regulation of lipid homeostasis, is upregulated in the liver of obese and diabetic mice, but the biological consequences of this induction are largely unknown. This study was aimed at further characterizing this upregulation and exploring the downstream biological effects of specific activators on hepatic lipid metabolism.

Methods: Hepatic expression of peroxisome proliferator-activated receptor-[gamma]1 and [gamma]2 mRNA and protein was analyzed by real-time polymerase chain reaction and Western immunoblotting in KKAy mice and ob/ob mice. KKAy mice were treated with thiazolidinediones, and hepatic triglyceride content and lipid distribution were analyzed biochemically and by histopathology.

Results: KKAy mice exhibited a marked increase in hepatic peroxisome proliferator-activated receptor-[gamma]1 mRNA and protein levels, whereas the [gamma]2 isoform was upregulated in ob/ob mice. Treatment of KKAy mice with troglitazone or rosiglitazone resulted in severe microvesicular periacinar steatosis, whereas lean control mice did not develop any pathological liver changes. Hepatic triglyceride levels, however, were not altered by the treatment.

Conclusions: In mice with obesity-associated upregulated hepatic peroxisome proliferator-activated receptor-[gamma] expression, thiazolidinediones may produce hepatic steatosis. Under pathophysiological conditions, such as non-insulin-dependent diabetes, the liver may thus become sensitized towards peroxisome proliferator-activated receptor-[gamma]-activating drugs.

Background/Aims: High levels of tumor necrosis factor-alpha are associated with an increased risk of severe encephalopathy in acute liver failure, and experimental studies suggest that tumor necrosis factor-alpha plays a role in the development of acetaminophen (paracetamol)-induced liver injury and associated multiple organ failure. Inter-individual variations in the production of tumor necrosis factor-alpha have been linked to genomic polymorphisms within the tumor necrosis factor-alpha locus. This study examined whether specific tumor necrosis factor polymorphisms are associated with variations in the severity of clinical features in acetaminophen-induced acute liver failure.

Methods: Genotypes at the -308 tumor necrosis factor A and tumor necrosis factor B Nco1 polymorphic sites were determined in 97 patients with severe acetaminophen-induced hepatotoxicity and 109 controls, using polymerase chain reaction and restriction fragment length polymorphism. The relationship between liver injury, multiple organ failure and encephalopathy, determined retrospectively from clinical notes and genotype, was examined.

Results: No significant association was found between either tumor necrosis factor A or B genotype and parameters for multiple organ failure or liver injury. The tumor necrosis factor B1B1 genotype was significantly under-represented in those patients developing severe encephalopathy (p = 0.03) and a multivariate logistic regression analysis confirmed the influence of tumor necrosis factor B genotype (pDRB1*03, which is closely linked to the TNFB locus).

Conclusions: The development of acute liver failure is unlikely to be primarily sepsis driven. However, the apparent protective effect of the tumor necrosis factor B1B1 genotype on the development of severe encephalopathy may be related to the effects of this genotype on tumor necrosis factor-alpha production in sepsis.


http://www.sciencedirect.com/science/article/B6W7C-4406K4G-18/2/67fadb284334668d9302953df3a736db4

Background/Aims: Primary sclerosing cholangitis is associated with the HLA haplotypes A1-B8-DRB3*0101-DRB1*0301-DQA1*0501-DQB1*0201 and DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603. However, the interpretation of these genetic associations is controversial. One explanation may be that HLA-encoded susceptibility is due to other genes carried on these haplotypes such as the HLA class III tumor necrosis factor genes. The aim of the study was to investigate tumor necrosis factor genetics in a large series of well-defined patients.

Methods: One hundred and ten HLA genotyped patients and 126 control subjects were studied by polymerase chain reaction genotyping for 3 different tumor necrosis factor gene polymorphisms: -308, -238 and an Nco1 restriction fragment length polymorphism in the lymphotoxin [alpha] gene.

Results: Overall, 58% of patients had the TNF2 allele, compared with 29% of controls, pc=0.0001. No association was found with either of the other tumor necrosis factor polymorphisms examined. TNF2 was significantly increased in the presence of B8 and DRB3*0101 only, and was independent of DRB1*0301 (pcB8 and TNF2 were stronger than the associations with any of the HLA class II alleles examined).

Conclusion: HLA-encoded genetic susceptibility to primary sclerosing cholangitis may be determined by polymorphism within the HLA class III region, in particular with the TNF2 allele.


http://www.sciencedirect.com/science/article/B6W7C-44FMP1S-WB/2/4901d2d9a29e243b1464970b6ca06c33

Background/Aims: Despite anti-HBs immunoglobulin therapy, hepatitis B virus (HBV) infection...
recurs in a high proportion of patients transplanted for HBsAg positive and serum HBV DNA negative chronic liver disease. The contribution of HBV genetic variability to disease recurrence has not been yet thoroughly addressed. We have therefore undertaken a detailed comparison of preS/S and preC/C sequences in two selected patients with recurrence of HBsAg and HBV DNA after transplantation. Methods: PreS/S and preC/C regions were amplified by PCR from the serum, peripheral blood mononuclear cell (PBMC) and liver tissues of two patients transplanted for end stage HBV-related cirrhosis. Samples were taken both pre- and post-transplantation. HBV-sequences from four to nine clones were determined and compared. Results: A mixing of different HBV DNA molecules was observed within and between serum, liver and PBMC samples. Sequences from both patients showed mutations in the preC region which abolished HBeAg secretion, and in the preS2 initiation codon which prevented preS2 envelope protein production. In addition, for both patients, deletions in the preS2 domains (3 and 21 base pairs) led to the expression of modified preS1 envelope protein. For one patient, the predominant HBs protein sequence found in the PBMC before transplantation showed four specific mutations. One of these mutations was in the "a" determinant (codon 144, asparagine to glycine change) of the major envelope protein. These mutations were not detected, as predominant mutations, in the liver and serum pre-orthotopic liver transplant samples. In contrast, after liver transplantation, this was the major form identified in serum, liver and PBMC. Conclusions: Our results have shown the selection of different HBV DNA molecules in liver and mononuclear cells. In addition, they provide direct evidence for the role of PBMC in the infection of liver grafts and support the hypothesis that infection of PBMC might lead to selection of HBV variants which would escape immune therapy. Finally, we provide in vivo evidence for reinfection of the liver by HBV particles lacking preS2 envelope protein expression.


http://www.sciencedirect.com/science/article/B6W7C-42NH34J-6/2/6bb712051a2bd979d431b9c6297f1848

Background/Aims: The actual prevalence of the main hemochromatosis (HFE) mutations in the Italian adult population and their phenotypic expression have not yet been established. This information is key to advocate a mass-screening program. Methods: Two thousand one hundred adults were tested for the C282Y/H63D HFE gene mutations by an automated genotyping assay as well as transferrin saturation (TS) and serum ferritin levels. Results: No homozygotes for the C282Y mutation were found. Heterozygosity for the C282Y mutation was 3.1%, while heterozygosity and homozygosity for the H63D mutation were 21.5% and 2.5%, respectively. TS was significantly higher in C282Y heterozygotes and H63D homozygotes as compared to wild-type individuals (P). Interestingly, of the HFE wild-type subjects 5.9% had a TS value above the 45% threshold. Conclusions: This study shows that (i) the predicted prevalence for C282Y homozygosity in Italy is 1:3900; (ii) the C282Y/H63D wild-type population has an increased baseline of iron parameters possibly due to genetic factors not linked to the C282Y/H63D mutations; (iii) since in the latter population the actual tissue iron burden cannot be assessed, phenotypic (TS) screening in Italy is not recommended until the true prevalence of all mutations in the HFE gene and in other hemochromatosis genes will be established.


http://www.sciencedirect.com/science/article/B6W7C-437YW4M4-
Background/Aims: A non-enveloped single-stranded DNA virus (TTV) was detected in Japanese patients with fulminant hepatitis (47%) and chronic liver disease of unknown etiology (46%) more frequently than in blood donors (12%). Subsequent studies, however, questioned the association of TTV with liver disease. We further investigated the role of this novel virus in liver diseases.

Methods: We tested 106 patients and 102 blood donors for TTV by polymerase chain reaction using conserved region primers.

Results: TTV DNA was found in 19 of 102 volunteer blood donors (18.6%) and in 27 of 106 patients with liver disease (25.5%): 10 of 28 chronic hepatitis B (35.7%), 9 of 28 chronic hepatitis C (32.1%) and 8 of 50 (16%) cryptogenic liver disease patients. Previous interferon treatment was not associated with a significantly lower prevalence of TTV infection. TTV prevalence was higher in patients with blood exposure (42.8%, 6/14) than in patients without risk factors (21.4%, 18/84). Four of five patients (80%) with HBV familial infection and without blood exposure were also TTV positive. Partial nucleotide sequences from 3 Italian isolates diverged more than 30% from the 2 prototype genotypes G1 and G2 and were 88% homologous to the recently described genotype G4.

Conclusions: G1 and G2 TTV are common in Italy and in the USA in liver disease patients and in blood donors. The prevalence is high in patients with blood exposure but also in subjects without risk factors; other routes of transmission should therefore be considered.


http://www.sciencedirect.com/science/article/B6W7C-436FVDT-53/2/76b838311dc7a942c8b80dc9581f18eb

Background/Aims: Several studies have shown that cholestatic recurrent hepatitis is associated with very high HCV RNA loads in liver transplant recipients. The aim of this study was to investigate whether a correlation exists between cholestatic hepatitis posttransplant and the population of viral quasispecies.

Methods: One hundred and nine serial sera samples were tested from 15 recurrent HCV patients. Four of these patients showed severe cholestatic recurrent hepatitis. 11 patients demonstrated non-cholestatic recurrent hepatitis post-transplant. Quasispecies were detected by RT-PCR amplification of the HVR1 followed by single-stranded conformational polymorphism analysis.

Results: Forty-one samples from four cholestatic patients were tested. All four patients showed very stable quasispecies patterns post-transplant. One cholestatic patient also showed a stable quasispecies band pattern following retransplantation, again associated with severe cholestatic hepatitis. Sixty-eight samples were tested from the 11 non-cholestatic patients. In contrast, these patients showed significantly more quasispecies bands than the cholestatic patients. The noncholestatic patients also displayed fluctuating band patterns post-transplant. Serial samples were tested after retransplantation in one non-cholestatic patient, with a fluctuating pattern again seen. There was a negative correlation between the HCV RNA load in serum and the number of quasispecies bands.

Conclusions: Stable hepatitis C viral quasispecies associated with persistently high viral load in post-transplant cholestatic hepatitis suggest that viral escape from immune pressures may play a role in the pathogenesis of this condition.


http://www.sciencedirect.com/science/article/B6W7C-437YSV7-1S/2/96c313c3c8eb02430d9f41c00eefc105
Background/Aims: Knockout mice lacking mdr2, the murine analogue of human MDR3 P-glycoprotein, develop chronic non-suppurative cholangitis. Recently, a deficiency in MDR3 messenger RNA (mRNA) has been reported in a subtype of progressive familial intrahepatic cholestasis. Thus, reduced MDR3 gene expression could be involved in human cholestatic liver diseases.

Methods: We developed a sensitive and specific reverse transcription/competitive polymerase chain reaction for the semiquantitation of intrahepatic MDR3 mRNA levels. Using this method we determined the MDR3 mRNA levels in 52 liver specimens from primary biliary cirrhosis (n=11), chronic hepatitis B (n=5), chronic hepatitis C (n=14), non-cholestatic cirrhosis (n=9) and controls (n=13).

Results: MDR3 mRNA was detected in all specimens with some variation in mRNA levels. No significant differences in the mean MDR3 mRNA levels were present between the groups studied, including normal controls.

Conclusions: We found no evidence for deficient or severely reduced intrahepatic MDR3 mRNA in primary biliary cirrhosis, nor were mRNA levels altered significantly by virus-induced inflammation or by cirrhosis. The reverse transcription/competitive polymerase chain reaction assay described here should be a useful tool in the semiquantitative study of MDR3 mRNA levels in small tissue specimens.


http://www.sciencedirect.com/science/article/B6W7C-436FX5D-GW/2/f987e66f0a0522691dd090bc3a6b0d71

Background/Aims: Primary biliary cirrhosis is a chronic cholestatic liver disease characterized by progressive inflammatory destruction of bile ducts, with eventual hepatic fibrosis and cirrhosis. Since primary biliary cirrhosis affects predominantly middle-aged women and has pathological similarities to hepatic graft-versus-host-disease, we investigated whether fetal cell microchimerism might be involved in the development of this disease.

Methods: The presence of Y-chromosome-specific sequences was analyzed by polymerase chain reaction using peripheral blood mononuclear cells from women with primary biliary cirrhosis (n=18) and healthy (control) women (n=18), and by in situ hybridization of liver biopsy sections from women with primary biliary cirrhosis (n=19) and women with chronic hepatitis C or alcoholic liver disease (n=20).

Results: Male cells were detected in liver biopsy specimens of 8 of 19 patients (42%) with primary biliary cirrhosis. Y-chromosome-containing cells were not seen in any of the liver biopsy specimens from women with chronic hepatitis C or alcoholic liver disease. Male cells were detected in peripheral blood mononuclear cells from one healthy control at a level of 1 male cell per 106 female cells, but were not detected in peripheral blood mononuclear cells of women with primary biliary cirrhosis.

Conclusions: The presence of male cells in the liver of women with primary biliary cirrhosis raises the possibility that fetal cell microchimerism may be involved in the pathogenesis of this chronic liver disease.


http://www.sciencedirect.com/science/article/B6W7C-44FMP1S-WF/2/101480370e9ff4ac1a0979f2865aee3

Background/Aims: The hepatotropic conjugate of adenine arabinoside monophosphate with lactosaminated poly--lysine (-Poly(Lys)) must have a high solubility in order to be injected in a small volume compatible with the intramuscular route. In this paper the molecular weights of Poly(Lys) which allowed the synthesis of conjugates with the properties of high solubility and limited loss by the kidney were determined and a procedure for obtaining Poly(Lys) preparations...
with the required range of polymerization has been described. Methods: Conjugates were prepared using Poly(Lys) of different molecular weights obtained by the procedure described here or purchased from a commercial source. Their solubility and renal loss in mice was determined. Results: Poly(Lys) with molecular weights ranging from 45000 and 65000 Da guarantees high solubility and low renal elimination of the conjugates. Conjugate preparations with these properties, intramuscularly administered to woodchuck hepatitis virus-infected woodchucks for 37 days at a daily dose of 5.8 mg/kg exerted a strong antiviral activity. These preparations were devoid of acute toxicity in rat and caused no toxic effects when injected intramuscularly daily for 28 days at a dose ten times higher than that active in woodchucks. Conclusions: The results support the possibility of a clinical use of Poly(Lys) to obtain liver targeting of adenine arabinoside monophosphate for the treatment of chronic hepatitis B virus infection.


http://www.sciencedirect.com/science/article/B6W7C-48W2GWT-2/2/4edf902a03cabbc9bf293dd1ba88fd7

Background/Aims: Recent advances in stem cell research have revealed that hepatic stem/progenitor cells may play an important role in liver development and regeneration. However, a lack of detectable definitive markers in viable cells has hindered their primary culture from adult livers. Methods: Enzymatically dissociated liver cells from green fluorescent protein (GFP)-transgenic mice, which express GFP highly in liver endodermal cells, were sorted by GFP expression using a fluorescence-activated cell sorter. Sorted cells were characterized, and also low-density cultured for extended periods to determine their proliferation and clonal differentiation capacities. Results: When CD45-TER119- side-scatterlow GFPhigh cells were sorted, [alpha]-fetoprotein-positive immature endoderm-characterized cells, having high growth potential, were present in this population. Clonal analysis and electron microscopic evaluation revealed that each single cell of this population could differentiate not only into hepatocytes, but also into biliary epithelial cells, showing their bilineage differentiation activity. When surface markers were analyzed, they were positive for Integrin-[alpha]6 and -[beta]1, but negative for c-Kit and Thy1.1. Conclusions: Combination of GFP-transgenic mice and fluorescence-activated cell sorting enabled purification of hepatic progenitor cells from adult mouse liver. Further analysis of this population may lead to purification of their human correspondence that would be an ideal cell-source candidate for regenerative medicine.


http://www.sciencedirect.com/science/article/B6W7C-42NH34J-R/2/a90f9ff7f1d5a3d1090c5c63d17e8854d

We report the unique occurrence of an allogenic bone marrow transplantation performed as the donor was suffering from an acute hepatitis A. The bone marrow was contaminated at the time of collection, as demonstrated by hepatitis A virus (HAV) RNA detected by RT-PCR. Hepatitis A virus infection in such a situation could have resulted in a severe liver disease in the recipient. However, although we could demonstrate that the recipient had been infected, he did not develop a symptomatic hepatitis A but only minor disturbances of liver function tests between days 35 and 55. Both the postponement of the transplantation and the use of intravenous polyvalent immunoglobulins have probably played a key role in decreasing the viral load and allowing a
A possible role of the grafted immune system might also be envisaged, as suggested by the de novo synthesis of IgM in the recipient.


Background/Aims: The determination of HCV-RNA concentration in liver samples is likely to provide interesting insights for the study of disease progression and for evaluation of the efficacy of anti-viral therapy. Methods: a procedure was developed for the precise quantification of HCV-RNA in liver biopsies, based on the competitive reverse transcription-polymerase chain reaction technology. This competitive assay consists of the co-amplification of the target RNA with known amounts of a competitor RNA molecule containing the same sequence as the target plus an insertion in the middle, allowing resolution of the two amplification products by gel electrophoresis. Results: The amounts of HCV-genomic-RNA and [beta]-actin mRNA (the latter being used as an internal standard to overcome the problem of reproducibility of quantitative RNA extraction) were evaluated in liver biopsies of 15 patients affected by hepatitis C virus-positive chronic liver disease at the time of diagnosis. All the patients underwent [alpha]-interferon therapy for 6 months and were subsequently followed for at least 1 further year after the end of treatment. Viral RNA concentration (which ranged from 2 to 2.7 x 10^5 HCV-RNA molecules per 10^16 [beta]-actin molecules) directly correlated with the efficacy of treatment, indicating that low levels of viral replication in the liver are associated with a poor response to therapy. Conclusions: This study suggests that the determination of viral load in the liver is an important prognostic tool for the prediction of the efficacy of [alpha]-interferon therapy.


Background/Aims: The analysis of hepatitis B virus (HBV) X protein genetic variability and is correlation with liver disease severity have only been addressed, so far, on whole liver extracts. We have studied, therefore, the HBV X protein (HBx) gene sequence in morphologically well-characterised tumour and non-tumour liver cells from patients with HBV-related hepatocellular carcinoma. Methods: Using laser capture microdissection (LCM), we picked up six to eight groups of tumour and non-tumour hepatocytes in serial frozen sections from six patients. After global DNA preamplification followed by HBx-specific polymerase chain reaction, the HBx gene was sequenced in each group of microdissected cells. We also validated the quantification of HBV-DNA in microdissected hepatocytes using HBV Amplicor(R). Results: Heterogeneous mutations in HBx gene were found in distinct cirrhotic nodules and tumour areas from the same patient. Mutations at aa 127, 130 and 131 were frequently detected but there was no distinct point mutation profile between tumour and non-tumour samples. In contrast, deletions in HBx gene, which were found in five/six patients, were more frequent in tumour-derived sequences (6/18) than in non-tumour-derived sequences (1/20). Conclusions: We have shown that LCM provides a direct insight of intrahepatic HBV infection. Using this technique, we demonstrated the persistence of distinct HBx encoding sequences in clonally expanding cells, thus supporting the hypothesis that HBx deletions may be implicated in liver carcinogenesis.

http://www.sciencedirect.com/science/article/B6W7C-4406K4G-13/2/b8f963051ddc0d0324b6135ac0bc3665

Background/Aims: An unenveloped single-stranded DNA virus (TTV) has been reported in association with elevated transaminase levels in patients with posttransfusion hepatitis and in those with acute or chronic liver disease of unknown etiology. To further evaluate the association of TTV with liver disease, TTV DNA was searched for in patients with acute or chronic liver disease of various etiologies. Methods: TTV DNA was determined by polymerase chain reaction with hemi-nested primers in 64 patients with acute or chronic liver disease of unknown etiology and in 100 with acute or chronic liver disease positive for antibody to hepatitis C virus (HCV) as well as HCV RNA. Results: TTV DNA was detected in two of the seven (29%) patients with acute hepatitis of unknown etiology, but in none of the four patients with acute HCV-associated hepatitis. It was detected in 27 of the 57 (47%) patients with chronic liver disease of unknown etiology at a frequency significantly higher (p Conclusions: Based on the obtained results, TTV has a role in the development of acute and chronic liver disease of unknown etiology.


http://www.sciencedirect.com/science/article/B6W7C-43S17MD-2X/2/bb055edd504415efac1e7b01728e2436

Background/Aims: In the liver, intrahepatic biliary cells are the sole site of expression of the cystic fibrosis transmembrane conductance regulator, the product of the cystic fibrosis gene. We examined the regulation of cystic fibrosis transmembrane conductance regulator gene expression by protein kinase C in the recently characterized human liver epithelial BC1 cell line which expresses, at early confluence, both biliary (cystic fibrosis transmembrane conductance regulator, cytokeratin 19) and hepatocytic (albumin) specific markers. Methods: Expression of the cystic fibrosis transmembrane conductance regulator was examined at the mRNA level by Northern blot, reverse transcription-polymerase chain reaction and nuclear run-on assays and at the protein level by Western bloting. The functionality of this protein was tested by measurement of chloride efflux. Protein kinase C isoform expression and cytosol-to-membrane translocation were analysed by Western blotting. Results: 1) Phorbol ester down-regulated cystic fibrosis transmembrane conductance regulator mRNA expression in a time- and dose-dependent manner through a post-transcriptional mechanism with concomitant inhibition of stimulated chloride efflux. 2) Phorbol ester also activated protein kinase C as indicated by the cytosol-to-membrane translocation of both protein kinase C [alpha] and [epsiv] the two major protein kinase C isotypes expressed by BC1 cells. 3) Further, maximal down-regulation of the cystic fibrosis transmembrane conductance regulator mRNA by the phorbol ester was inhibited by H7 and by GF 109203X, two known protein kinase C inhibitors. Conclusions: These findings provide the first evidence for phorbol ester-induced down-regulation of cystic fibrosis transmembrane conductance regulator mRNA expression in a human liver epithelial cell line and point to a role for the classical protein kinase C [alpha] and the novel protein kinase C [epsiv] in this process.

Background/Aims: In order to explore the role of cytokines in the pathogenesis of liver cirrhosis, we analyzed their gene expression in hepatic biopsies from patients with alcoholic liver cirrhosis, post-hepatitis C liver cirrhosis, and idiopathic portal hypertension without cirrhosis.

Methods: We assessed the gene expression of interleukins 1[beta], 2, 6, 8, and 10, as well as of tumor necrosis factor-[alpha], transforming growth factor-[beta] and interferon-[gamma] by a quantitative polymerase chain reaction.

Results: We found high levels of transforming growth factor-[beta] in post-hepatitis C liver cirrhosis, high to moderate in alcoholic liver cirrhosis and low in non-cirrhotic specimens. Expression of interleukin-10, tumor necrosis factor-[alpha], and interferon-[gamma] genes was detected in most post-hepatitis C liver cirrhosis, but not in idiopathic portal hypertension or alcoholic liver cirrhosis biopsies. The interleukin1-[beta], 6 and 8 gene expression was significantly lower in alcoholic liver cirrhosis compared to post-hepatitis C liver cirrhosis, but higher compared to idiopathic portal hypertension specimens. Thus, post-hepatitis C liver cirrhosis samples showed a high degree of cytokine gene expression, whereas in alcoholic liver cirrhosis it tended to be moderate, and restricted to some cytokines (transforming growth factor-[beta], interleukin-1, 6 and 8, but not interleukin-10, tumor necrosis factor-[alpha] or interferon-[gamma]). In contrast, most non-cirrhotic specimens showed a restricted and low cytokine gene expression.

Conclusions: These data suggest that transforming growth factor-[beta] may have an important role in liver fibrosis and inflammation. Interleukin-1[beta], 6, 8, tumor necrosis factor-[alpha] and interferon-[gamma], appear to participate in the pathogenesis of the mild to severe inflammatory phenomena seen in alcoholic and post-hepatitis C liver cirrhosis, respectively. Our data suggest that tumor necrosis factor-[alpha] does not participate in the hepatocellular damage of alcoholic liver cirrhosis, and indicate that neither interferon-[gamma] nor interleukin-10, at least at the levels observed in post-hepatitis C liver cirrhosis, are able to counteract the fibrotic/inflammatory process seen in this condition.


Background/Aims: Gene transfer using recombinant Moloney murine leukemia viruses (rMoMuLV) requires mitosis of the target cell. Previously, we and others have used partial hepatectomy for induction of hepatocellular proliferation for gene transfer to the liver in vivo by exsanguineous perfusion with rMoMuLV. We hypothesized that induction of hepatocellular proliferation by combined administration of two hepatocellular mitogens, hepatocyte growth factor (HGF) and triiodothyronine (T3), should permit rMoMuLV-mediated gene transfer into liver without invasive approaches.

Methods: HGF (1 mg/kg) was perfused continuously into the portal vein of Wistar male rats and T3 (2 mg/kg) was injected subcutaneously. Twenty-four hours after injecting HGF and T3, the state of proliferation of hepatocytes was estimated from the incorporation of 5'-bromo-2'-deoxy-uridine (BrdU). The amphotropic retroviral receptor (Ram-1) expression of liver was evaluated at different time points after injecting HGF and T3 by means of Northern blotting using Ram-1 cDNA probe. In order to evaluate the role of hormone treatment on gene transfer, the liver was perfused exsanguineously with rMoMuLV 24 h after injection with hormones.

Results: Rats treated with a combination of HGF and T3 expressed BrdU and [beta]-galactosidase in 8.3% and 0.7% of hepatocytes, respectively. On the other hand, there was near absence of gene transfer in untreated rats perfused with rMoMuLV. Twenty-four hours after the initial manipulation, abundant expression of Ram-1 mRNA was observed in rat hepatocytes treated with HGF plus T3.

Conclusions: Stimulation of hepatocellular mitosis and upregulation of
Ram-1 expression by HGF and T3 augment retrovirus-mediated gene transfer into hepatocytes.


http://www.sciencedirect.com/science/article/B6W7C-436FV7M-3G/2/4bd283149a68f4d8d9f609b2f3f969d

Background/Aims: In the majority of cases of fulminant "viral" hepatitis in Australia, no known aetiological agent can be isolated. We have examined the possible role of the recently discovered hepatitis G virus (HGV) in such cases. Methods: An HGV specific reverse transcription polymerase chain reaction (RT-PCR) was performed on pre- and post-liver transplant serum from 14 patients who were referred for transplantation at our unit between 1989 and 1995 for unexplained fulminant hepatic failure. Eleven patients successfully underwent transplantation and three died while waiting for a suitable donor organ. Hepatitis viruses A-E were excluded by standard serological and PCR based testing. HGV RT-PCR was also performed on 21 other, randomly selected, liver transplant recipients ("controls"). Results: The 14 fulminant cases were HGV RT-PCR negative prior to transplantation while five of 21 controls were positive. Post-transplant, eight of the 11 fulminant patients were found to be HGV RT-PCR positive and the same five controls remained HGV RT-PCR positive. In three of the eight fulminant patients the HGV infection resolved. Conclusions: Our data indicate that HGV infection is unlikely to be responsible for fulminant hepatitis and that it is probably acquired from blood and/or blood products during the transplantation process. Furthermore, long-term carriage of HGV post-transplant is not associated with clinically apparent liver disease.


http://www.sciencedirect.com/science/article/B6W7C-48TK824-1/2/9b169755f3212b6bea9e5d6e71f3b

Background/Aims: The prognosis of hepatocellular carcinoma (HCC) is poor because of frequent intrahepatic metastasis (IM) or multicentric carcinogenesis (MC). We compared the effectiveness of loss of heterozygosity (LOH) analysis in the diagnosis of these two forms with that of histopathological diagnosis. Methods: Using LOH analysis of 15 specific DNA microsatellite loci, tumor clonality was assessed in 37 cases. Results: LOH was observed in 30% of seven solitary tumors. According to these results, the selected threshold to diagnose MC was a difference in the LOH status at more than 30% of the analyzed loci, when comparing two samples in the same liver. In nine multiple HCCs, identical genetic and histopathological diagnoses were found in four (IM: 2, MC: 2). Of 21 recurrent tumors, 19 showed LOH for at least one marker. IM and MC were genetically diagnosed in five and ten patients, respectively. Genetic and histopathological diagnoses were identical in ten of 19 patients (IM: 5, MC: 5). Five genetic MC were histopathologically diagnosed as IM (3) and 'undetermined' (2). Conclusions: Genetic diagnosis by LOH analysis may be more strict and specific than histopathological diagnosis in the differential diagnosis of IM and MC.

Background: Non-Wilsonian hepatic copper toxicosis includes Indian childhood cirrhosis (ICC), endemic Tyrolean infantile cirrhosis (ETIC) and the non-Indian disease known as idiopathic copper toxicosis (ICT). These entities resemble the hepatic copper overload observed in livers of Bedlington terriers with respect to their clinical presentation and biochemical and histological findings. We recently cloned the gene causing copper toxicosis in Bedlington terriers, MURR1, as well as the orthologous human gene on chromosome 2p13-p16.

Aim: To study the human orthologue of the canine copper toxicosis gene as a candidate gene for ICC, ETIC, and ICT.

Methods: We sequenced the exons and the intron-exon boundaries of the human MURR1 gene in 12 patients with classical ICC, one patient with ETIC, and 10 patients with ICT to see whether these patients display any mutations in the human orthologue of the canine copper toxicosis gene.

Results: No mutations in the MURR1 gene, including the intron-exon boundaries, were identified in a total of 23 patients with non-Wilsonian hepatic copper toxicosis.

Conclusions: Our results demonstrate that copper toxicosis in Bedlington terriers is not an animal model for the non-Wilsonian hepatic copper toxicosis described in this study.


Background/Aims: A novel virus, designated the TT virus (TTV), was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology, in Japan. Subsequently, TTV was suggested to be a causative agent in a proportion of cases with cryptogenic hepatitis in Japan. This study aimed to elucidate the significance of TTV infection in cases with cryptogenic liver disease in Korea, a neighbor of Japan.

Methods: The prevalence of TTV infection was studied in 120 patients with liver diseases, including 85 patients diagnosed as having non-B, non-C liver diseases. As controls, 220 blood donors were also examined. TTV DNA was detected by polymerase chain reaction, and the sequence was analyzed by phylogenetic analysis.

Results: Fourteen (14.0%) of 100 accepted blood donors, 23 (19.2%) of 120 rejected blood donors, and 15 (17.6%) of 85 patients with non-B, non-C liver diseases were positive for TTV DNA. The prevalences of TTV infection among these groups were not significantly different. Phylogenetic analysis suggested the existence of four major genotypes of TTV. The proportions of each genotype among patients with non-B, non-C liver diseases were not different from those among accepted blood donors.

Conclusions: TTV exists in Korea, but the prevalence among patients with non-B, non-C liver diseases was almost the same as that among blood donors. TTV may not be the main causative agent of cryptogenic liver disease in Korea. The relationship between non-B, non-C liver diseases and TTV genotype remains unclear, although TTV can be classified into four genotypes.


Background/Aims: The roles of c-met proto-oncogene and hepatocyte growth factor in human livers have not been shown.

Methods: Gene expressions of both c-met and hepatocyte growth
factor were quantified in livers with chronic active hepatitis and in cirrhotic livers with hepatocellular carcinoma as well as in normal controls, using competitive reverse transcription polymerase chain reaction. Results: C-met expression was significantly increased in chronic active hepatitis compared with control livers, and c-met expression in chronic active hepatitis correlated with serum alanine aminotransferase levels. Hepatocyte growth factor expression was increased in some patients with chronic active hepatitis compared with controls, and there was a significant correlation between c-met expression and hepatocyte growth factor expression. On the other hand, in hepatocellular carcinoma tissues, c-met expression was increased in some cases, while that in the surrounding non-carcinomatous tissues was similar to normal controls. Hepatocyte growth factor expression was not detected in the hepatocellular carcinoma tissues and was low in the surrounding non-carcinomatous tissues. Conclusions: These findings suggest that hepatocyte growth factor may be involved in the regeneration of hepatocytes via paracrine mechanism in chronic active hepatitis, while the regulation of c-met expression in hepatocellular carcinoma tissues may be independent of hepatocyte growth factor stimulation.


http://www.sciencedirect.com/science/article/B6W7C-44FMKNF-1M/2/66d608c117602c5e5d32f7dc48040dad

Background/Aims: Survival after orthotopic liver transplantation for hepatocellular carcinoma is limited by a high rate of tumor recurrence. A polymerase chain reaction assay based on the detection of albumin mRNA expression in peripheral blood for detection of hematogenous micrometastasis of hepatocellular carcinoma has been described, which may help to select candidates for orthotopic liver transplantation. Methods: The prognostic value of a highly sensitive nested reverse transcription-polymerase chain reaction assay was evaluated in comparison with the TNM-classification of the International Union against Cancer in a population of liver transplant candidates. Results: Eighty patients with liver disease and 42 control patients were evaluated. Six of 21 patients with hepatocellular carcinoma and 11 of 59 patients with other diseases of the liver were positive for albumin reverse transcription-polymerase chain reaction, making this assay an indicator of ongoing liver damage without absolute specificity for hepatocellular carcinoma. Twelve patients with hepatoma were followed after liver transplantation and seven of those patients had a tumor recurrence within 12 months. Six of these patients with recurrence had International Union against Cancer stage IV A tumors preoperatively, while only one of them was positive for albumin reverse transcription-polymerase chain reaction before transplantation. Only one patient with a stage I to III tumor had a recurrence within 12 months. Conclusions: Detection of albumin mRNA in peripheral blood by reverse transcription-polymerase chain reaction seems to be an unreliable marker for assessing hematogenous spread of hepatocellular carcinoma. With International Union against Cancer stage IV A being a much better predictor of tumor recurrence, the practical value of albumin mRNA reverse transcription-polymerase chain reaction for patient selection in liver transplant candidates seems to be very limited.


http://www.sciencedirect.com/science/article/B6W7C-436FX5D-HJ/2/7fc8e501a128c1f11d235ac6d8add1c6

Background/Aims: Hepatitis E virus (HEV) is an enterically transmitted pathogen that appears sporadically in non-endemic countries. We studied HEV as a causal agent of acute hepatitis
cases in the Spanish population, and the role of pigs as an animal reservoir. Methods: The presence of HEV-RNA was analysed by nested polymerase chain reaction in 37 serum samples from patients with acute viral hepatitis, 48 porcine serum samples, 6 pig faecal samples and 12 slaughterhouse sewage samples. Presence of antibodies was also tested in porcine sera. Results: HEV-RNA was found in 3 human serum samples from patients presenting IgG anti-HEV antibodies. Nucleotide sequence analysis identified 2 strains with 93.4% identity, phylogenetically most closely related to the Greece1 isolate, and more closely related to North American and other European strains than to those from endemic regions. HEV-RNA was also detected in slaughterhouse sewage mainly from pigs, presenting 92-94% nucleotide similarity compared to the strains detected in the human sera. Twenty-five per cent of the pigs tested presented IgG anti-HEV antibodies. Conclusions: These data suggest that the HEV could be more widespread than previously thought, and present new evidence of the close relationship between HEV strains detected in pigs and those from acute hepatitis patients.


Background/Aims: Lamivudine is an antiviral drug that is used to treat hepatitis B virus (HBV) infection. Long-term therapy does not completely suppress viral replication, and resistant mutants emerge. Resistance is mediated by changes in the tyrosine-methionine-apartate-aspartate (YMDD) motif in the catalytic site of the HBV polymerase gene. We describe a method to detect and quantify mutant viral populations using amplification refractory mutation system (ARMS) PCR. Methods: We developed a real-time ARMS-PCR to detect point mutations in the polymerase gene. Using real-time PCR (LightCycler) with a ResonSense probe, PCRs were performed using clones of the HBV polymerase gene containing the different YMDD mutations. Dilution series of the templates were made and tested against each of the primer pairs. This method was applied to quantify mutant virus in patient serum samples. Results: As little as 0.01% mutant DNA in 105-109 copies wild-type DNA were detected. The method is more sensitive than amplicon sequencing, which is the current method of mutant determination in the YMDD motif. Conclusions: This study demonstrates a rapid, highly sensitive and reproducible method of quantifying mutant HBV virus in lamivudine treated patients. It can be used to monitor patients before and during lamivudine therapy.


Background/Aims: To clarify the association between the reovirus infection of the hepatobiliary tree and the development of infantile obstructive cholangiopathy (IOC) including biliary atresia (BA) and congenital dilatation of the bile duct (CBD). Methods: We designed reovirus common primers for nested RT-PCR based on the L3 gene segment. The spectrum and the sensitivity of common primers were evaluated with purified reoviral RNAs and reovirus mixed with stool samples. Then, nested RT-PCR were performed with hepatobiliary and fecal samples obtained from patients with BA, CBD, and control diseases. Additionally, electron microscopy of stool samples was performed. Results: The L3 common primers could amplify cDNAs synthesized from RNAs of three prototypes of reovirus, and detect as much as 5.0 x 103 plaque forming unit of serotype 3 Dearing strain in 100 mg of fecal samples. However, no amplification product was
detected in 136 hepatobiliary tissues taken from 67 patients including 26 BAs and 28 CBDs, or in 65 fecal samples obtained from 15 patients including 10 BAs and 1 CBD. Additionally, viral particles were not found in any stool specimens by the electron microscope. Conclusions: These data do not suggest that reoviruses play a major role in the etiology of IOC or BA.


http://www.sciencedirect.com/science/article/B6W7C-49KS3D7-2/2/12fab404a39726e0729693863ac028df

Background/Aims: CD103, a mucosal integrin [alpha]E[beta]7, binds to E-cadherin expressed on hepatocytes and bile duct epithelium in the liver. Although CD103+ T cells are enriched in intestinal intraepithelial lymphocytes, the localization of those cells in the liver is unknown. Methods: We investigated whether CD103+ cells are present in human livers, and how they are associated with the intrahepatic development of T cells by flow cytometry and immunohistochemistry. Results: Human livers contain significantly (P+ cells in CD4+ and CD8+ T cells (25.7+/=-13.5 and 27.1+/=-19.3%, respectively) than peripheral blood lymphocytes. Moreover, CD103+ cells in the liver, but not in peripheral blood, contained T cells with intermediate expression level of T cell receptor [alpha][beta]. Those cells consist of mostly CD4+ and CD4-CD8- cells, and expressed low level of CD56 and interleukin-2 receptor beta chain in most of the population. These characteristics are distinct from natural killer T cells, which have been thought to be extrathymic T cells in human livers. Moreover, intrahepatic CD103+ cells expressed mRNA for recombination-activating gene-1, -2 and pre T cell receptor-alpha detected by reverse transcription-polymerase chain reaction. Conclusions: CD103+ T cells are preferentially accumulated in human livers, and those T cells show characteristics of extrathymic T cells.


http://www.sciencedirect.com/science/article/B6W7C-4DX25H6-2/2/d3cda20c05f3841b4415070e239965c

Background/Aims: NAD(P)H: quinone oxidoreductase 1 (NQO1) and glutathione S-transferase P1 (GSTP1) belong to phase II xenobiotic-metabolizing enzymes. GSTP1 inactivation via CpG island hypermethylation in hepatocellular carcinoma (HCC) was previously reported, but the involvement of NQO1 in HCC is not well known. In this study, we assessed the transcription and status of methylation of NQO1 gene in human hepatoma cells and primary human HCC tissues. Methods: NQO1 transcription and DNA hypermethylation in hepatoma cells with or without 5-aza-deoxycytidine (5-Aza-CdR) treatment were investigated by reverse-transcription PCR (RT-PCR), sodium bisulfite sequencing and methylation-specific PCR (MSP). The methylation status of NQO1 and GSTP1, and NQO1 mRNA in 44 HCC cases was also analyzed by MSP and real-time PCR, respectively. Results: NQO1 transcription was down-regulated and the CpG island DNA was hypermethylated in Hep3B and HuH6 cells. After treatment with 5-Aza-CdR, NQO1 transcription was restored and CpG island DNA was demethylated in these cells. MSP analysis revealed that NQO1 hypermethylation occurred in 50.0% of HCC. All of the tumors that exhibited lesser amounts of NQO1 mRNA than corresponding non-tumorous tissues showed NQO1 hypermethylation. Conclusions: NQO1 transcription might be inappropriately suppressed by promoter hypermethylation in a subset of HCC, as well as GSTP1 gene.

http://www.sciencedirect.com/science/article/B6W7C-437YTX1-58/2/4be075b047733ff704d1380c1f9a9cdf

Background/Aims: A variety of data suggest that microbial infections and, in particular, atypical mycobacteria infections, may either initiate and/or be associated with the pathogenesis of primary biliary cirrhosis.Methods: To address this hypothesis, use was made of polymerase chain reaction techniques and primers specific for the 16s rRNA gene of Eubacteria, Archaeabacteria, Mycobacteria and Helicobacter to determine if such sequences were detectable in liver tissue specimens from 29 patients with primary biliary cirrhosis. Similar liver tissues from patients with primary sclerosing cholangitis, chronic hepatitis, alcoholic liver disease and otherwise normal donors were analyzed in parallel. Genomic DNA was extracted from each of these liver tissue specimens using sterile techniques to avoid possible laboratory contamination. The DNA was subjected to polymerase chain reaction amplification using bacterial genus specific primers and the amplified products cloned and sequenced. Sequence data were analyzed by searching for homology to existing genes.Results: Sequences from primary biliary cirrhosis and control livers corresponded to those found in a variety of bacteria, but no consensus sequence was found in primary biliary cirrhosis specimens. Neither Archaeabacteria nor Mycobacteria products were detected in liver specimens of patients with primary biliary cirrhosis, and Helicobacter pylori DNA was detected in only one primary biliary cirrhosis patient.Conclusions: Although bacterial infection, particularly with intracellular organisms, has been suggested to play a role in the initiation of primary biliary cirrhosis, there is no evidence from this study to suggest an ongoing chronic infectious process.


http://www.sciencedirect.com/science/article/B6W7C-436FWR9-DC/2/8d13f2dd3719242a1a238650ffba72f

Background/Aims: The prevalence and pathogenicity of TT virus, recently identified in patients with non A-non G post-transfusional hepatitis, are questioned.Methods: We investigated the impact of this new viral infection in a large series of patients with non A-non G, cryptogenic, non-viral and viral-related, acute and chronic liver diseases (n=577) and blood donors (n=300). TTV DNA was detected in serum by heminested polymerase chain reaction. Phylogenetic analysis was performed in 13 isolates.Results: TTV DNA was detected in 6/25 and 15/127 patients with cryptogenic non A-non G acute and chronic liver disease, respectively. TTV DNA positive subjects with post-transfusional acute hepatitis scored negative before transfusion. TTV prevalence was increased in patients with cryptogenic non A-non G acute and chronic liver disease, respectively. TTV DNA positive subjects with post-transfusional acute hepatitis scored negative before transfusion. TTV prevalence was increased in patients with cryptogenic non A-non G acute and chronic liver disease compared to blood donors (6/300; ppConclusions: These results support the view that TTV is a widely spread infectious agent with a weak pathogenicity. It raises the possibility, however, that TTV might be implicated in a few cases of acute and chronic non A-non G hepatitis. TTV-DNA-analysed sequences are related to genotypes 1 and 2 described in Europe.


http://www.sciencedirect.com/science/article/B6W7C-49CTBJP-5/2/aa5518c2bd177a427f2fe3c9f6f4e93
Background/Aims: This study using primary-cultured rat hepatic stellate cells (HSCs) was aimed to reveal the effect of carbenoxolone and the other gap-junction blockers on the proliferation and activation of HSCs. Methods: HSC morphology was microscopically evaluated. DNA synthesis was determined by [3H]thymidine incorporation. Expression of HSC activation markers and cell cycle-related proteins was evaluated by Western blot. Collagen [alpha]1(I) mRNA expression was evaluated by quantitative reverse transcription polymerase chain reaction. Results: Carbenoxolone triggered the morphological change of activated HSCs without inducing apoptosis. Culture-induced DNA synthesis was suppressed to 22.6 and 8.51%, respectively, by 40 and 80 [mu]M carbenoxolone. The other gap-junction blockers failed to affect the morphology and the DNA synthesis of activated HSCs. Carbenoxolone decreased the expression of cyclins D1/2 and cyclin-dependent kinases 4/6. Platelet-derived growth factor (PDGF)-BB-elicited DNA synthesis was reduced to 45.6 and 3.27%, respectively, by 40 and 80 [mu]M carbenoxolone. Phosphorylation of c-Raf, MEK and mitogen-activated protein kinase, but not PDGF receptor [beta], under PDGF-BB stimulation was attenuated by carbenoxolone. Collagen [alpha]1(I) mRNA expression was significantly reduced. In addition, carbenoxolone suppressed the activation process of quiescent HSCs. Conclusions: Carbenoxolone reduced the DNA synthesis and the expression of collagen [alpha]1(I) mRNA in activated HSCs independently of its pharmacological action as gap-junction blocker.


http://www.sciencedirect.com/science/article/B6W7C-44B6RS5-2/2/3bf748c5024e47ba1cffe7bc829ee049

Background/Aims: In hepatitis C infection, the production of inappropriate cytokine levels appears to contribute to viral persistence and to affect the response to antiviral therapy. Additionally, polymorphisms in the cytokine genes may affect the production of the cytokines. In this study, we determined the frequency of the genotypes associated with polymorphisms of the interleukin-10 and tumor necrosis factor-[alpha] gene promoters, and transforming growth factor-[beta]1 gene leader sequence, and investigated their association with clinical features and the response to interferon-[alpha] and ribavirin therapy in chronic hepatitis C infection. Methods: Genomic DNA from 80 patients and 37 racially matched healthy controls was studied by polymerase chain reaction and direct automated sequencing. Results: The interleukin-10 -1082 G/G genotype was identified more frequently in patients than in controls (P=0.048). The transforming-growth-factor-[beta]1 +29 (codon 10) C/C genotype was associated with resistance to the therapy (P=0.029). After adjusting for potential confounding variables, patients exhibiting the C/C genotype were less likely to respond to treatment than patients with the T/T or T/C genotypes. Conclusions: These results suggest that inheritance of the interleukin-10 -1082 G/G and the transforming growth factor-[beta]1 +29 C/C genotypes, which appear to affect the cytokine production, may be associated with susceptibility to chronic hepatitis C infection and resistance to combined antiviral therapy.


http://www.sciencedirect.com/science/article/B6W7C-4BDVY20-1/2/1e7a74500676dc4c2f83924dc53e3c80

Background/Aims: Debate continues on whether serum and intrahepatic HCV viral loads are correlated and if HCV viral load correlates with the severity of liver disease. These difficulties may
at least in part be linked to liver cell heterogeneity, when total liver extracts from HCV-infected individuals are tested for HCV RNA quantification. We have therefore investigated the feasibility of quantifying HCV replication using a laser-based microdissection technique. Methods: We compared the results with those obtained for serum HCV RNA quantification and immunochemistry in the case of HCV antigen detection in the liver. Twenty-one HCV-positive patients with chronic active hepatitis (n=10) or cirrhosis (n=11) were analyzed. Results: A positive correlation (P=0.0019) was observed between HCV RNA quantifications in sera and microdissected cells. Immunohistochemistry demonstrated that HCV antigen hepatocytes were randomly distributed within liver lobules. Their percentage varied in different patients (0-40%), but did not correlate with the HCV viral load. Conclusions: We have designed a sensitive methodology to evaluate the intrahepatic HCV viral load by combining a standardized RNA quantification method with microdissected hepatocytes from frozen liver needle biopsies. Our results directly demonstrate a positive correlation between serum and intrahepatic viral loads, which therefore provides a reliable reflection of intrahepatic HCV replication.


http://www.sciencedirect.com/science/article/B6W7C-436FWWH-F3/2/0460c8b1435c3048239ab1de7e6d51c

Background/Aims: Liver fibrosis is a dynamic state between matrix production and degradation. Since our report in 1974, many studies have examined collagenase and liver fibrosis, but not the identification of cells responsible for collagenase production in vivo. The aim of this study was to investigate the gene expression of interstitial collagenase in the progressive and recovery phases of experimental rat liver fibrosis by in situ hybridization. Methods: We examined the gene expression of interstitial collagenase (MMP-13) in the progressive and recovery phase of experimental rat liver fibrosis induced by chronic CCl4 intoxication by reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization. In order to identify the cells expressing MMP-13 mRNA by in situ hybridization, immunohistochemistry was performed using serial sections. Results: In normal rat liver, a faint band for MMP-13 mRNA was observed by RT-PCR, but not by in situ hybridization. The livers of rats treated with CCl4 for 4 weeks showed fatty metamorphosis but no definite fibrosis. Positive signals for MMP-13 mRNA were observed in scattered mesenchymal cells, within lobules which seem to be stellate cells from immunohistochemical staining. Once the fibrosis became prominent, the faint band for MMP-13 mRNA was detected only by RT-PCR and very few signals, if any, by in situ hybridization. On the other hand, in the recovery phase of liver fibrosis, gene expression of MMP-13 was markedly enhanced. Strong positive cells by in situ hybridization were observed mainly at the interface between the resolving fibrous septa and the parenchyma. Overlapping both images of in situ hybridization and immunohistochemical staining with the help of a computer revealed that some positive cells, but not all cells, were stellate cells stained with [alpha]-smooth muscle actin antibody. Conclusions: MMP-13 participates in the degradation of newly-formed matrix in the recovery from rat liver fibrosis more than in the remodeling of extracellular matrix for the formation of fibrosis. Hepatic stellate cells play a crucial role in MMP-13 production in the recovery from fibrosis, though not all stellate cells were positive for MMP-13 mRNA. Further investigation into gene expression of MMP-13 in recovery will lead to new strategies for the treatment of liver cirrhosis.

Background/Aims: Fas transduces apoptotic signals upon cross-linking with the Fas ligand, which is experimentally replaced by anti-Fas antibodies. Because little is known about Fas expression and function in hepatocellular carcinoma, these issues are addressed in the current article.

Methods: We examined Fas expressions at protein and mRNA levels, and susceptibility to anti-Fas-mediated apoptosis, on six hepatocellular carcinoma cell lines.

Results: Two cell lines constitutively expressed high levels of Fas both on their cell surface and in their cytoplasm, whereas the other four cell lines expressed Fas mainly in their cytoplasm. Fas mRNA of normal size was detected in all cell lines in reverse transcriptase-polymerase chain reaction analyses.

Although a Fas mRNA variant, suggesting soluble Fas molecule, was detected in the two cell lines expressing high levels of Fas, its amount was very small compared to that of normal-sized Fas transcript. Anti-Fas dose-dependently induced apoptosis exclusively in the two cell lines which constitutively express high levels of cell surface Fas. However, after preincubation with interferon-\[gamma\], one cell line with low surface Fas expression became anti-Fas sensitive equivalent to the two cell lines expressing surface Fas at high levels. Studies of two clonally related cell lines showed that dedifferentiated clones had lower Fas expression and resistance to anti-Fas, suggesting deterioration of Fas system after clonal cell dedifferentiation.

Conclusions: These findings suggest sensitivity to anti-Fas is virtually relevant to cell surface Fas, but not to cytoplasmic Fas expression. However, its expression level does not correlate to sensitivity to anti-Fas.


Background/Aims: The aims were to study: 1) the prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic liver disease, 2) viral and host immunogenetic factors that may predispose to diabetes, and 3) liver transplantation outcome in patients with or without diabetes.

Methods: Fasting blood glucose values of patients who underwent liver transplantation because of hepatitis C-related cirrhosis (73 patients) were compared with those of patients with cirrhosis due to cholestatic (78 patients) or alcoholic liver disease (53 patients) and to a general population. Data on diabetes prevalence in a population without liver cirrhosis was based on the prevalence of diabetes in Olmsted County, Minnesota, residents. HLA was determined using serologic assays. Hepatitis C virus genotypes were determined with polymerase chain reaction amplification and direct sequencing. Hepatitis G RNA was detected with polymerase chain reaction. Liver transplantation outcome in patients with or without diabetes was determined with rejection, retransplantation, or death at 1 year after transplantation as end points.

Results: Of 64 patients with hepatitis C alone, 16 (25%) had diabetes before transplantation compared with 1 of 78 (1.3%) with cholestatic liver disease \( (p=0.0001) \) and 10 of 53 (19%) with alcoholic liver disease \( (p=0.36) \). Nine patients had hepatitis C plus cholestatic liver disease; one of these (11%) had diabetes. The prevalence of diabetes in patients with cholestatic liver cirrhosis was not different from that of the general population. The frequency of hepatitis G virus coinfection, HLA-DR3, or HLA-DR4 in hepatitis C and diabetes was not different from that of hepatitis C alone. The distribution of hepatitis C virus genotype was similar in those with and those without diabetes. Diabetes was not associated with increased risk of rejection, retransplantation, or death at 1 year after transplantation, and had no impact on overall survival after transplantation.

Conclusions: 1) The risk of diabetes is not increased in patients with liver cirrhosis due to cholestatic liver disease but is in patients with liver cirrhosis due to hepatitis C or alcoholic liver disease; 2) cofactors (age, sex, body mass index, hepatitis G virus...
coinfection, hepatitis C virus genotype, or HLA-DR3/DR4) did not explain the increased risk of diabetes in patients with hepatitis C; 3) diabetes before liver transplantation did not change the outcome at 1 year after transplantation or survival.

Journal of Hospital Infection  (4)


http://www.sciencedirect.com/science/article/B6WJP-4CDJ0YX-8/2/be18b13d8fc4a465721b243c12f7b9b4

Enterococci are frequently isolated as nosocomial pathogens and have often acquired intrinsic drug resistances. Molecular typing techniques have been developed to assist in epidemiological and infection control measures. This study investigates enterococci with high-level aminoglycoside resistance (HLAR) from the National University Hospital (NUH) of Singapore, and evaluates and compares three methods for typing: restriction enzyme analysis by conventional gel electrophoresis [restriction fragment length polymorphism (RFLP)], pulsed-field gel electrophoresis (PFGE), and polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD). Fifty-two isolates of Enterococcus faecalis and 13 isolates of Enterococcus faecium were used for the study. The numbers of patterns obtained for E. faecalis and E. faecium were 26 and 4, respectively by the RFLP method, and very similar discrimination was obtained by PFGE. RAPD PCR results were not reliably reproducible. A single pattern type by RFLP accounted for 16 of the E. faecalis isolates, suggesting hospital spread.


http://www.sciencedirect.com/science/article/B6WJP-4C7633Y-1H/2/9fca7908a67e0478a30d86b22390fc03

In this study we investigated the epidemiology of a cluster of cutaneous infections owing to Aspergillus niger, which occurred in neutropenic patients in a bone marrow transplant unit. Heavy environmental contamination with the mould was found in the ward kitchen adjacent to the unit. The clinical and environmental isolates were typed by random amplification of polymorphic DNA (RAPD), which showed one of the patients was infected with the same strain as that isolated repeatedly from the kitchen area. In another case, contaminated stockinette material was implicated as the source of infection. Thorough cleaning of the ward kitchen resulted in no further cases on the unit. This highlights the fact that aspergilli may spread to patients by air, food or other vehicles, and underlines the importance of searching for a source and ensuring high levels of hospital hygiene are maintained.

Isolates of Salmonella Enteritidis from 81 patients from Herlev Hospital or from Copenhagen County were analysed by random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and phage-typing. Fourteen polymorphic markers from five decamer primers unambiguously placed all isolates into six RAPD groups: 65 isolates of phage-type 6 (PFGE type I) were resolved into three RAPD groups constituting 86, 12, and 2%, respectively. A fourth RAPD group of 10 isolates was coincident with phage type 8 (PFGE type II) and two isolates, one phage-type 1, the other phage-type 4 (both PFGE type I) formed the fifth group. The sixth group of four isolates was not phage typeable and was PFGE type III. Forty outbreak-related isolates of phage-type 6 were resolved into three strains. No diversity of phage-type 6 was found among isolates unrelated to the outbreak. It is concluded that RAPD is useful as a tool in investigations of microbial outbreaks in its own right, or to supplement phage-typing and PFGE of Salmonella Enteritidis.


Universal or 'broad-range' eubacterial polymerase chain reaction (PCR) was performed on 53 isolates from environmental water-associated sites in a haematology unit (N=22), and the outer surfaces of cleaning lotion containers sited throughout a tertiary referral hospital (N=31). 16 S rDNA PCR was performed using two sets of universal primers, including the novel reverse primer, XB4, to generate a composite amplicon of 1068 bp, which was sequenced to obtain each isolate's identity. Sequence analysis was able to identify 51 isolates. Most (75% from the haematology unit and 81% from cleaner containers) were Gram-positive. Nine different genera were identified from the haematology unit and 13 from the cleaning lotion containers. This study provides the first reports of Terrabacter spp. and Brachybacterium paraconglomeratum isolated from a hospital environment. As unusual and difficult-to-identify environmental organisms are unlikely to be clinically significant, and molecular identification is costly and labour-intensive, we recommend that molecular methods are only used as an adjunct to first-line phenotypic identification schemes where a definitive identification is required. Where molecular identification methods are justified, partial 16 S rDNA PCR and sequencing employing the novel universal primer XB4, is a valuable and reliable technique.

Journal of Infection 6

Objectives. To study the action of factors produced by living Campylobacter jejuni (C. jejuni) against those present within sonicated and filtrated bacteria on induction of potential cytokines by the human intestinal cell line INT407. Methods. We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis. Results. The data herein display dissociation of cytokine profiles induced on by living C. jejuni. Exposure of INT407 cells to 10^6 live bacteria showed the highest numbers of cytokine producing cells of all examined cytokines. IFN-\([\gamma]\) was the highest induced cytokine followed by IL-10, TNF-\([\alpha]\) and lastly IL-4. Also, abrogation of induction of the proinflammatory cytokines IFN-\([\gamma]\) and TNF-\([\alpha]\) but not the antiinflammatory cytokines IL-4 and IL-10 by sonicated and filtrated bacteria was depicted. At the mRNA level, TNF-\([\alpha]\) signals were noted in accordance with its protein levels since increased TNF-\([\alpha]\) mRNA signals were registered only after stimulation with living bacteria. Very low or no induction of TNF-\([\alpha]\) was registered with non-stimulated cells. Conclusions. These results illustrate for the first time a role for factors from living bacteria in directing the immune response towards Th1 type. Characterization of such factors may be essential for future immunotherapeutic interventions during severe bacterial infections.


Objectives. To develop a sensitive multiplex PCR to detect HCMV, HHV6 and HHV7, to test this PCR on urine specimens sent to the virus diagnostic laboratory and on stored urine samples from HIV-positive patients and their HIV-negative partners and to compare the sensitivity of the multiplex PCR with the diagnostic laboratory's routine service for the detection of HCMV. Study design. Primers specific for each of the three viruses were combined in a multiplex PCR that was then optimised for sensitivity. This PCR was applied prospectively to 413 unselected routine urine specimens over a 1 year period and retrospectively to 258 urine specimens from 63 HIV-positive patients and 10 HIV-negative partners. Methods. In the prospective study, the multiplex PCR detected 40 specimens positive for HCMV alone, 10 for HHV6, 3 for HHV7 and 3 with a dual infection of HCMV and HHV6. The sensitivity for HCMV was 93.5% by multiplex PCR compared to 28.3% by culture. HHV6 DNA was detected in 6 neonates (2-21 days) and HHV7 DNA in 2 neonates (4 and 20 days). In the retrospective study of HIV patients, HCMV was the most commonly detected virus (55.6%) compared to HHV6 (7.9%) and HHV7 (4.8%). Conclusions. The multiplex PCR was significantly more sensitive than non-DNA based procedures for the detection of HCMV. Urine may be a useful non-invasive specimen for the detection of HHV6 and HHV7 and their presence in neonates suggest perinatal transmission or the possibility of in utero infection.


http://www.sciencedirect.com/science/article/B6WJT-4BDM2K7-1/2/cc5d7040b208975927e4039ca0875a22

Objectives. To investigate the production of dynamic [alpha] and [beta] chemokines represented by interleukin-8 (IL-8) as [alpha] chemokine and CCL2 (monocyte-chemoattractant protein-1,
CCR2 ligand), CCL4 (macrophage-inflammatory protein-1[beta], CCR5 ligand), CCL3 (macrophage-inflammatory protein-1[alpha], CCR1/5 ligand), (CCL5, regulated upon activation, normal T-cell expressed and secreted (RANTES, CCR5 ligand) as [beta] chemokines by the human intestinal cell line INT407 stimulated with factors produced by living Campylobacter jejuni (C. jejuni) and those present within sonicated and filtrated bacteria.Methods. We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis.Results. Living bacteria induced increased numbers of IL-8, CCL4 and CCL2 but not CCL3 or CCL5 producing cells. Low numbers of IL-8, CCL4 and CCL2 producing cells were detected with filtrated supernatant compared to living and sonicated bacteria. A non-significant low number of chemokine producing cells was noted when comparing numbers of chemokine producing cells stimulated with living C. jejuni to those stimulated with sonicated bacteria, indicating that the triggering factors involved in stimulation with living bacteria were still active after sonication, but they were largely lost upon filtration. The mRNA signals for IL-8 were noted in conformity with its protein levels as increased IL-8 mRNA signals were registered after stimulation with living and sonicated bacteria but not with filtrated supernatant.Conclusions. Preferential production of chemokines probably induced by membrane associate factors of C. jejuni acting on intestinal epithelial cells is presented. These chemokines are suggested to be part of an inflammatory network affecting cell types that contribute to initiation and/or resolution of the infection.


ObjectiveThe goal of this study was to evaluate the prevalence of influenza A virus on surfaces in day care and home settings to better assess the potential role of fomites in the transmission of influenza.MethodsDuring two and a half years, 218 fomites were tested from 14 different day care centers. Ten different fomites from bathrooms, kitchens and play areas were sampled. In addition, 92 fomites from eight different homes with children were tested over 6 months. Fourteen different household fomites from bathrooms, kitchens and living areas were sampled. Influenza A viral RNA was detected using reverse transcriptase-polymerase chain reaction.ResultsInfluenza was detected on 23% of day care fomites sampled during the fall and 53% of fomites sampled during the spring. Spring and fall sample data was determined to be statistically different at the 0.05 [alpha]-level by Chi-square analysis PP=0.00002. There was no statistical difference found between moist and dry fomites (Chi square P=0.13998). No influenza was detected on home fomites sampled during the summer. In contrast, influenza was detected on 59% of home fomites sampled during March.ConclusionsInfluenza A virus was detected on over 50% of the fomites tested in homes and day care centers during influenza season.


http://www.sciencedirect.com/science/article/B6WJT-48S4KR9-1/2/a2e902a2e243a0ac54f03fdff8e41d71

An 89-year male with pyrexia and suspected bacteremia was admitted to hospital, where a Gram-negative rod was identified from blood culture. The organism was difficult to identify phenotypically and the resulting sequencing of a 559 bp section of the 16S rRNA gene did not have a high homology score (>97.0%) with any deposited GenBank accession numbers and hence was not able to be assigned to a species within any genus. Given that the isolate was a member of the alpha subclass of the Proteobacteria but did not fall into any of the known genera
with more than 93.7% homology (Brucella, Rhizobium, Ochrobactrum, Agrobacterium), we believe this isolate to represent a novel [alpha]-proteobacterium, which was the cause of bacteraemia in this patient.


http://www.sciencedirect.com/science/article/B6WJT-4C604VR-1/2/0be762e87f0f3e339a3778882e328e39

Objective. The emergence of multidrug resistance within Streptococcus pneumoniae population was analysed, correlating penicillin resistance Pen(R) with secondary antibiotic resistance, capsular serotype, and genetic diversity among isolates.

Methods. DNA fingerprinting, following macro-restriction enzyme digestion and pulse field gel electrophoresis (PFGE), and restriction fragment analysis of the PBP 2b gene, following PCR amplification, were performed on the Pen(R) S. pneumoniae, among 377 clinical isolates obtained from the clinical microbiology laboratory (University of Michigan Medical Center).

Results. Overall 35% of the isolates were Pen(R) of which 45% demonstrated high-level penicillin (Pen(R)-R, MIC>1). Respiratory isolates were more likely to be Pen(R) (ppConclusion. The emergence of multidrug resistance in the S. pneumoniae population in SE Michigan is not due to expansion of a single or limited number of resistant clones, is occurring most frequently in the paediatric population and is associated with a decreased susceptibility to penicillin.

Journal of Inorganic Biochemistry (2)


We have studied the ability of several bioorganometallic clusters \( [(\mu\text{-H})\text{Os}_3(CO)_9(L)(\mu_3-\eta_2-(Q-H))] \), where \( L = [P(C_6H_4SO_3Na)_3] \) or \( [P(OCH_2CH_2NMe_3I)_3] \), and \( Q = \text{quinoline, 3-aminoquinoline, quinoxaline or phenanthridine, of inhibiting telomerase, a crucial enzyme for cancer progression. In general, quinolines have shown interesting biological properties, especially in inhibiting enzymes. For example, the 2,3,7-trichloro-5-nitroquinoxaline (TNQX) exhibited strong anti-telomerase activity in vitro. Among the quinoline-clusters under study, only the negatively charged ones (by virtue of the sulfonated phosphines) exhibited good anti-telomerase activity on semi-purified enzyme in a cell-free assay, while they were ineffective in vitro on Taq, a different DNA-polymerase. On the contrary, the treatment of breast cancer MCF-7 cell line did not evidence any activity of these clusters, suggesting a low aptitude for crossing cell membrane. Furthermore, all clusters exhibited non-specific, acute cytotoxicity, probably due to accumulation on cell membranes by virtue of their amphiphilic character. A detailed study of Os uptake and accumulation in MCF-7 cells supported this hypothesis.\)
An in vitro selection was carried out with Zn2+ to isolate novel RNA molecules, zinc-dependent aptamers, that bind to HIV-1 Tat protein. RNAs bound to Tat were collected by using a nitrocellulose filter from a library of random RNAs and regenerated to the next generation of the RNA library by subsequent reverse transcription, polymerase chain reaction, and transcription. Sequences of the selected RNAs were determined after 6 and 12 rounds of the selection. The control clones after normal selection procedure with Mg2+ had a consensus UUG that resembled essential sequences of TAR or Arg aptamers. On the other hand, many unique sequences were revealed from a library selected with Zn2+ and the RNA with most abundant sequence (clone 31) bound to Tat tightly only when Zn2+ existed. The secondary structure of clone 31 RNA was predicted by using a computational prediction with our thermodynamic parameters and enzymatic scission of the RNA. Several model RNAs were prepared and the binding property of these RNAs to Tat were investigated. As a result, all the model RNAs did not reproduce the binding property of clone 31. Therefore, the Tat aptamer that acts with Zn2+ should require a relatively longer region of the sequence which is able to offer tertiary cooperation of several motifs for the binding.

Gene expression during cold acclimation at a moderately low temperature (15[deg]C) was studied in Drosophila melanogaster using a subtraction technique. A gene homologous to senescence marker protein-30 (SMP30), which has a Ca2+-binding function, was up-regulated at the transcription level after acclimation to 15[deg]C. This gene (henceforth referred to as Dca) was also expressed at a higher level in individuals reared at 15[deg]C from the egg stage than in those reared at 25[deg]C. Moreover, DCA mRNA increased at the senescent stage in Drosophila, although SMP30 is reported to decrease at senescent stages in mammals. In situ hybridization to polytene chromosomes revealed that the Dca gene was located at 88D on chromosome 3R. The 5' flanking region of this gene had AP-1 (a transcription factor of SMP30) binding sites, stress response element and some other transcription factor binding sites. The function of DCA was discussed in relation to the possible regulation of cytosolic Ca2+ concentration.

We examined the larvae of several organophosphate and pyrethroid-resistant Mexican strains of Boophilus microplus using biochemical and molecular tests to investigate the mechanisms conferring acaricide resistance. The electrophoretic profiles of esterase activity in protein extracts from coumaphos and permethrin-resistant strains compared to the susceptible strain revealed distinct differences, which inhibitor studies attributed to carboxylesterases. Esterase hydrolysis assays showed significant enhancement of both total and permethrin hydrolysis in one pyrethroid-resistant strain, with no enhancement in two other strains with very high resistance to pyrethroids. Sequence analysis of sodium channel mRNA fragments in all pyrethroid-resistant strains determined that they did not possess the classic kdr and super-kdr mutations known to confer pyrethroid resistance in several insect species. Using reverse transcriptase-polymerase chain reaction (RT-PCR) with degenerate primers designed from conserved regions of insect esterase amino acid sequences, a B. microplus larval cDNA fragment was isolated whose deduced amino acid sequence was significantly similar to esterases from a wide range of species. In Northern blot RNA analysis the cDNA hybridized to a 2.1 kb mRNA that was abundant in all resistant strains except one, in which a very low abundance could provide a marker for the mechanism conferring resistance in this strain.

The objective of this research was to identify a reliable biochemical indicator for diapause (dormancy) in the boll weevil, Anthonomus grandis. Hemolymph polypeptides from reproductive and diapausing weevils were compared using denaturing sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). A 77-kDa protein, which proved to be a hexamerin (AgSP-1), strongly correlated with morphological diapause characters in both male and female adult weevils. N-terminal sequence analysis identified the first 25 amino acids of the mature
protein and was used to develop an antibody to AgSP-1. Anti-AgSP-1 reacted only with hemolymph from diapausing weevils of both sexes but not with hemolymph from reproductive weevils. Also, the yolk protein, vitellogenin (VG), inversely correlated with AgSP-1. Juvenile hormone regulates VG synthesis in most insect species. Juvenile hormone is reported to stimulate reproductive maturation in the boll weevil (Physiological Entomology 22 (1997) 261) and to be absent during diapause (Physiological Entomology 22 (1997a) 269). Therefore, the juvenile hormone (JH) mimic, methoprene, was used to assess the role of JH activity in the boll weevil for terminating diapause, stimulating reproductive maturation and possibly influencing AgSP-1 titers. Application of methoprene was not effective in activating reproductive development. Hemolymph from methoprene-treated, females contained VG and AgSP-1 titers that were similar to acetone-treated and untreated control weevils. Using a genomic DNA library and 3' RACE, two clones were isolated that yielded the complete sequence of AgSP-1 as well as a portion of the 5' untranslated region. Northern blot analysis confirmed the presence of a 2.5 kB transcript for AgSP-1 in the fat body of diapausing weevils. AgSP-1 was also present in the fat body of reproductive weevils, but to a lesser extent. No sex-related differences in gene expression were observed; diapausing weevils of both sexes showed similar levels of AgSP-1 expression. An inverse correlation was observed between the VG transcript and AgSP-1 mRNA. VG was highly expressed in the fat body of reproductive females and only slightly expressed in tissue from diapausing females. Our data suggests that AgSP-1 is a diapause-specific protein in adult weevils and that JH, alone, is not effective in terminating diapause.


http://www.sciencedirect.com/science/article/B6T3F-3VKS2SP-1/2/955600051653b8c6360eaee42deb6832

Differential cDNA display and quantitative RT-PCR suggested that the riboflavin synthase complex of the aphid endosymbiont, Buchnera, is active only when the symbiotic system is maintained and well organized in young hosts. Since this finding suggested the provision of riboflavin by Buchnera, we examined the effect of dietary riboflavin on the performance of symbiotic and aposymbiotic aphids using chemically-defined diets. Our results indicate: (1) dietary riboflavin is slightly detrimental to young, symbiotic aphids; (2) dietary riboflavin is essential to aposymbiotic aphids; (3) dietary riboflavin remarkably improves the performance of aposymbiotic aphids. These results strongly suggest that young, symbiotic aphids are provided with riboflavin by their endosymbionts, Buchnera.

Journal of Invertebrate Pathology


http://www.sciencedirect.com/science/article/B6WJV-47VYRMD-2/2/bafbe1ad0fb428b62ee1878b121e06d2
Field studies on the efficacy and persistence of an introduced strain of Beauveria bassiana for insect control require detection assays to differentiate the non-native strain from indigenous populations. In this study we developed strain-specific molecular markers based on polymerase chain reaction amplification of sequence-characterized amplified regions (SCAR) in combination with dilution plating on semi-selective medium to detect and estimate density of propagules of a commercial strain of B. bassiana (strain GHA) in field samples. Using random amplified polymorphic DNA (RAPD) analysis, unique fragments that distinguished GHA from other strains of B. bassiana were obtained. Three amplicons, OPA-140.44, OPA-150.44, and OPB-90.67, generated with RAPD primers were cloned and sequenced and used as bases for designing SCAR primers OPA14 F/R445, OPA15 F/R441, and OPB9 F/R677, respectively. All three SCAR primers were highly sensitive, capable of detecting 100 pg B. bassiana GHA genomic DNA, and thus could be used to detect varying levels of the fungus in the field.


http://www.sciencedirect.com/science/article/B6WJV-4DS94TM-2/2/a5dcf8e07ccbd314422934e2d71d3898

This is the first report of Thelohania solenopsae infections in monogyne (single-queen) Solenopsis invicta colonies in the field. In a 0.2-ha plot near Baton Rouge, Louisiana, inter-colony prevalence was 63% infection in June, 1999, when the population was 100% monogyne. In February, 2000, 21% of 33 monogyne and 90% of 10 polygyne colonies were infected. By May, 2001, the polygyne colonies had disappeared and only one of 34 monogyne colonies was infected, the final detection of T. solenopsae in the plot. Colony size did not differ significantly among the four types (monogyne versus polygyne X infected versus uninfected).


http://www.sciencedirect.com/science/article/B6WJV-47G3RXM-5/2/85e5f47319c2de0120a327d067ff6d0d


http://www.sciencedirect.com/science/article/B6WJV-49J8V7T-1/2/3fb5ce019e231e8e44b794d4da04c844

Hawaiian Tetragnatha spiders inhabiting small forest fragments on the Big Island of Hawaii are parasitized by mermithid nematodes. This is the first report of mermithid nematodes infecting spiders in Hawaii, and an initial attempt to characterize this host-parasite interaction. Because immature mermithids were not morphologically identifiable, a molecular identification was performed. A phylogenetic analysis based on 18S small ribosomal subunit nuclear gene sequences suggested that Hawaiian spider mermithids are more closely related to a mainland presumptive Aranimemis species that infects spiders, than to an insect-infecting mermithid collected on Oahu, HI, or to Mermis nigrescens, also a parasite of insects. Measured infection
prevalence was low (ranging from 0 to 4%) but differed significantly among forest fragments. Infection prevalence was associated significantly with fragment area, but not with spider density nor spider species richness. Results suggest that mermithid populations are sensitive to habitat fragmentation, but that changes in infection prevalence do not appear to affect spider community structure.

Journal of Laboratory and Clinical Medicine (8)


http://www.sciencedirect.com/science/article/B6WJW-4DW8K9J-D/2/6aa41bcd30b2a10d3c6aed5a14feade47

We report on a new mutation (4337T-->C) in exon 28 of the von Willebrand factor (VWF) gene, resulting in a substitution of L with P at residue 1446 (L1446P) of pre-pro-VWF. The defect is transmitted as a dominant trait and induces a reduced VWF synthesis, an abnormal VWF multimer pattern and a deficient VWF-platelet glycoprotein Ib interaction. The proband had low plasma and platelet VWF antigen levels, a reduced VWF collagen-binding capacity, and a disproportionately low VWF ristocetin cofactor activity, associated with the absence of ristocetin-induced platelet aggregation. Multimer analysis showed that the smaller multimers were slightly low, whereas the larger ones were significantly reduced or absent, with a clear cutoff between the two patterns. Similar hemostatic findings were observed in the proband's sister and nephew. Desmopressin administration restored VWF levels to near normal, but this was not so for VWF ristocetin cofactor activity or ristocetin-induced platelet aggregation. VWF multimers improved after desmopressin, moreover, with the larger forms restored and the smaller ones still relatively more represented. Recombinant P1446 VWF synthesis was reduced at heterozygous level, and its multimer pattern was similar to that observed in plasma VWF. These findings confirm the role of L1446P mutation in determining the von Willebrand disease (VWD) phenotype observed in our patients. Given the lack of large and intermediate VWF multimers, and the fact that the VWF-platelet interaction defect appears to be partially independent of multimer pattern, the VWD associated with L1446P mutation may belong to the type 2A/2M VWD variant.


http://www.sciencedirect.com/science/article/B6WJW-4BHJ5WF-B/2/ce9dc20cfac369f9eb6ffce6024ae4ac

The expression of inducible nitric oxide synthase (iNOS) expression and release of nitric oxide (NO) from macrophages are markedly increased in granulomatous infections. Activation of macrophages 1[alpha]-hydroxylase results in an increase of 1[alpha],25-dihydroxyvitamin D3 [1,25(OH)2D3]. However, the significance of this increased production is not completely understood. In this study, we analyzed 1,25(OH)2D3 and NO production in patients with tuberculosis infection and hypercalcemia and used lipopolysaccharide (LPS) to stimulate RAW
264.7 cells in an attempt to assess iNOS expression and gaseous NO production regulated by 1,25(OH)2D3. Peroxynitrite (OONO-) production and lactate dehydrogenase activity were also examined. Without additional stimulation, peripheral-blood mononuclear cells (PBMCs) from patients with tuberculosis converted more 25-hydroxyvitamin D3 to 1,25(OH)2D3 than did those from normal controls. These PBMCs released less NO than did those from control subjects, at baseline and in the stimulated state. We found that 1,25(OH)2D3 dose-dependently inhibited iNOS messenger RNA expression of the LPS-stimulated RAW 264.7 cells and also significantly reduced the gaseous NO release and OONO- production. Paralleling the 1,25(OH)2D3-induced inhibition of NO release were reductions in OONO- and LDH production. In conclusion, 1,25(OH)2D3 inhibited iNOS expression and reduced NO production by LPS-stimulated macrophages in the range of physiological doses. Inhibition of the NO surge was coupled with a reduction in OONO- and LDH production. Increased 1,25(OH)2D3 production and decreased release of NO from the PBMCs of patients with tuberculosis and hypercalcemia were also noted. We propose that 1,25(OH)2D3 production by macrophages may protect themselves against oxidative injuries caused by the NO burst. In the case of tuberculosis infection, increased 1,25(OH)2D3 synthesis may further contribute to the development of an unwanted phenomenon--hypercalcemia.


Heparin-induced thrombocytopenia (HIT) is a prothrombotic disorder caused by heparin-dependent IgG (HIT-IgG) that recognizes a complex of heparin and platelet factor 4 (PF4), leading to platelet activation via the platelet Fc[gamma]Rlla receptors (Fc[gamma]Rlla). Not all patients who generate HIT-IgG in response to heparin develop HIT, however, possibly because of observed differences in the ability of platelets from healthy individuals to be activated by HIT sera. It is known that a polymorphism in the platelet Fc[gamma]Rlla plays an important role in determining platelet reactivity to murine platelet-activating monoclonal antibodies of the IgGl subclass: homozygous arg131 ("high responder" or HR) platelets respond well, and homozygous his131 ("low responder" or LR) platelets respond poorly, respectively, to these murine monoclonal antibodies. We sought to determine whether the differing risk for HIT among patients who receive heparin, as well as the variable platelet reactivity to HIT sera, could be explained by preferential activation by HIT-IgG of platelets bearing a particular Fc[gamma]Rlla phenotype. We found that the LR Fc[gamma]Rlla gene frequency was significantly overrepresented among 84 HIT patients, compared with that of 264 control subjects (0.565 versus 0.471; p = 0.03). We studied the subclass distribution of HIT-IgG against its major antigen, heparin/PF4 complexes, and found that 55 of 61 (90%) HIT sera expressed IgG1 antibodies either alone (n = 47) or in combination with IgG2 (n = 5) or IgG3 (n = 3). We then compared the platelet-activating profile of HIT sera with murine platelet-activating monoclonal antibodies. As expected, the murine IgG1 monoclonal antibodies preferentially activated platelets from homozygous HR individuals. In contrast, however, the LR homozygous platelets exhibited the greatest reactivity to HIT sera that contained predominantly anti-heparin/PF4 antibodies of the IgG1 subclass. We conclude that the significant overrepresentation of the LR (his131) gene among patients with HIT may be explained by the preferential activation of LR Fc[gamma]Rlla platelets by HIT antibodies of the IgG1 subclass, which is the predominant immunoglobulin subclass generated in HIT.

Multiple infection by different hepatitis C virus (HCV) genotypes may be of great clinico-pathologic interest. In this study we determined the effective prevalence of coinfections by two or more HCV genotypes in 213 subjects with HCV-positive chronic hepatitis by using genotype-specific polymerase chain reaction (PCR), genotype-specific probe hybridization, and direct sequencing. The most prevalent genotype was HCV-1b (54%). HCV-2 (a/c) was also prevalent (27%), and types 1a and 3a were found in 5% and 3% of patients, respectively. A mixed infection was detected in 23 patients (10.8%): 4 out of 23 were coinfected by types 1a + 1b, while the remaining 19 patients had a 1b + 2 (a/c) mixed infection. Further analysis based on restriction fragment length polymorphism (RFLP) on type-specific PCR products was used to verify genotyping results. Only four coinfections (1a + 1b in 2 patients and 1b + 2 (a/c) in the remaining 2 patients, respectively) were confirmed by enzyme cleavage. All patients with true coinfection had long-lasting infection and liver cirrhosis. Both true and false mixed infections resulting from RFLP analysis were confirmed by direct sequencing of type-specific amplification products. We also determined a recurrent C/T transversion at position 618 in all sequenced samples. In 4 cases another point mutation (G/A at position 626) was found, reducing the number of mismatches between HCV-2 and HCV-1b from 4 to 3 (or 2). Interestingly, all HCV-2 isolates sequenced showed the highest degree of nucleotide homology with HCV-2 subtype c, confirming the relatively high prevalence of this subtype in Italy. In conclusion, we showed the possibility of multiple infection by different HCV types in the general population of chronically infected patients without particular risk factors, even if in a low percentage of cases. Further studies are needed to assess the clinical relevance of chronic HCV infection with multiple genotypes.


The platelet glycoprotein complex [alpha]IIb[beta]3 is required for platelet-fibrinogen binding and platelet aggregation. This study was designed to characterize the nucleotide sequence of the canine platelet [beta]3 gene from cDNA. The nucleotide and deduced amino acid sequences of the canine [beta]3 gene were 92% and 96% homologous, respectively, with the sequences previously established for the [beta]3 gene of human beings. Within the [beta]3 gene, the nucleotide sequence of cDNA prepared from canine platelets shared homology of 89% for the cytoplasmic domain, 93% for the transmembrane domain, 92% for the extracellular domain, 94% for the arginine-glycine-aspartic acid (RGD) binding domain, and 97% for the region associated with Ca2+-dependent stabilization of the [alpha]IIb[beta]3 fibrinogen-binding pocket. The deduced amino acid sequence of canine [beta]3 was 100%, 97%, 96%, and 95% homologous with the cytoplasmic, transmembrane, extracellular, and RGD-binding domains, respectively, and was 100% homologous with the region associated with Ca2+-dependent stabilization of the [alpha]IIb[beta]3 fibrinogen-binding pocket of [beta]3 in human beings. The canine platelet cDNA signal peptide segment of the [beta]3 gene encodes for 22 amino acids, as compared with 26 amino acids previously reported for human beings. The deduced amino acid sequence of canine [beta]3 corresponds to the high-frequency allelic form for five of the six alloantigenic sites reportedly associated with human platelets: Leu33Leu40Pro407Arg489Arg636. The apparent amino acid residue in position 143 (Pen alloantigen) of canine platelet [beta]3 is histidine.
compared with arginine in human beings. Knowledge of the \([\beta]3\) gene nucleotide sequence of normal dogs will facilitate the understanding of platelet \([\alpha]Ib[\beta]3\) structure-function relationships.


http://www.sciencedirect.com/science/article/B6WJW-4CMYYM6-6B/2/aff53ced3b61ae3864713866e629d4440

Peritoneal macrophages (PMOs) are important components of the host defense against microbial infection in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Incubation of human PMOs with cell-free supernatant (BFS), prepared from Staphylococcus aureus, inhibited prostaglandin E2 (PGE2) and thromboxane B2 (TXB2) production. Slot-blot analysis of cyclooxygenase-1 (Cox-1) and Cox-2 demonstrated a decrease in both Cox-1 (29%) and, to a greater extent, Cox-2 (65%) protein expression after BFS stimulation. When competitive polymerase chain reaction (PCR) was used, the peak levels of Cox-1 and Cox-2 messenger ribonucleic acid (mRNA) in unstimulated PMOs were 0.304 +/- 0.13 pmol/L and 9.61 +/- 2.84 pmol/L (mean +/- SEM, N = 3), respectively. After exposure of samples to BFS for 30 minutes, the level of Cox-2 mRNA was reduced to 0.59 +/- 0.449 pmol/L (16-fold reduction, p p < 0.05). In contrast, these same PMOs showed an increased expression of IL-6 mRNA and increased secretion of IL-6 protein. These results indicate that prostaglandin production in PMOs is regulated by alterations in both immunoreactive Cox-1 and Cox-2. The down-regulation of Cox metabolism in these cells is primarily related to the delayed and depressed increase in the Cox-2 gene product.


http://www.sciencedirect.com/science/article/B6WJW-4CN5N73-V/2/54bef7858315321dca6e69e8f1934558

Multiple cyst formation with fluid retention is a characteristic structural abnormality in polycystic kidney disease (PKD). Na/K adenosine triphosphatase (ATPase) is a major transporting membrane protein that is ubiquitous in the epithelial cell, which has been thought to be involved in cystogenesis. We have investigated the molecular and histologic basis of Na/K ATPase activity in experimental PKD in vivo. Rats were treated with diphenylthiazole (100 mg/100 gm body weight), and cyst formation was examined histologically. Na/K ATPase activity was measured enzymatically by using a fluorometric method, and reverse transcription-competitive polymerase chain reaction (RT-PCR) analysis was used to quantitate mRNA levels in the isolated single nephron segment. Kidneys were immunostained with subunit-specific antibodies to determine the localization of Na/K ATPase in the epithelial cell. The enzyme activity increased in the cortical collecting duct from 25.9 +/- 3.5 mmol/Lpmol/mm/min to 72.9 +/- 6.8 pmol/mm/min and in the outer medullary collecting duct from 13.0 +/- 3.9 mmol/Lpmol/mm/min to 58.5 +/- 9.8 pmol/mm/min (n = 6, p < 0.01); however, all other segments showed no significant changes. No significant alternation in [\(\alpha\)]- and [\(\beta\)]-subunits of Na/K ATPase mRNA levels was observed by competitive PCR assay in either segment. The enzyme was stained at the basolateral membrane even in the cystic tubules. Na/K ATPase activity was up-regulated in the cyst-formed kidney, but this was not accompanied with transcriptional up-regulation. Increased Na/K ATPase activity at normal locations may play a role in abnormal net fluid transport in the development and progression of experimental PKD.

http://www.sciencedirect.com/science/article/B6WJW-45SJFPK-1J/2/ea3abf60689aa421b51873c1c9140b92

The [alpha]-thalassemias are common genetic disorders that arise from reduced synthesis of the [alpha]-globin chains. At present, large-scale carrier screening and clinically valuable antenatal detection programs have not been established for the congenital disorder [alpha]-thalassemia ([alpha]-thal). We have developed a simple nonradioactive polymerase chain reaction (PCR) approach that can detect and differentiate several common [alpha]-globin gene deletional [alpha]-thals regardless of the break points. When three primer sets were used-two gene-specific sets for the [alpha]1- and [alpha]2-globin genes and one set for the [beta]-actin gene (serving as an internal control)-PCR products from genomic DNA were simultaneously amplified and analyzed after coamplification and gel electrophoresis. The number of [alpha]-globin genes present in the subjects was determined by the intensity of [alpha]1 and [alpha]2 bands normalized with that of [beta]-actin when using densitometry. Our results demonstrate that five common genotypes of deletional [alpha]-thal are differentiated by the ratios of [alpha]1/[beta]-actin and [alpha]2/[beta]-actin. We also examined the feasibility of coupling this allele-specific amplification to a color-complementary assay. This easy and reproducible PCR assay is suitable for identifying [alpha]-thal carriers in screenings of large populations and improving genetic counseling. (J Lab Clin Med 2001;137:290-5)

Journal of Molecular and Cellular Cardiology (7)


http://www.sciencedirect.com/science/article/B6WK6-47S6CWX-2/2/17362036670bae78358631ef196759ac

Lysophosphatidic acid (LPA) is a phospholipid messenger, which is released from activated platelets and leukocytes. This study examined the effects of LPA on myocardial contractility and characterized the signal transduction pathway involved in these effects. Functional effects of LPA were determined in isolated, electrically driven human myocardial preparations and rat cardiac myocytes. In human atrial and ventricular myocardial preparations, LPA (100 [mu]mol/l) decreased isoprenaline (0.03 [mu]mol/l) enhanced force of contraction by 17 +/- 2% and 28 +/- 3%, respectively. The effect of LPA was attenuated by suramin (1 mmol/l). In isolated rat cardiomyocytes, LPA (1-100 [mu]mol/l) concentration dependently abolished isoprenaline (0.03 [mu]mol/l) induced increase in cell shortening. This antiadrenergic effect was blunted after pretreatment with pertussis toxin (5 [mu]g/ml, 12 h). Forskolin (10 [mu]mol/l) stimulated adenylyl cyclase activity was inhibited by LPA in human myocardial membranes. PCR analysis of human atrial and ventricular cDNAs revealed the expression of two cognate LPA receptors: EDG-2 and EDG-7. Our results suggest that LPA exerts antiadrenergic effects on force of contraction in human and rodent myocardium via a G[alpha]i/o protein-mediated mechanism, most probably by LPA binding to the mammalian LPA receptors EDG-2 and/or EDG-7. This newly discovered
action of LPA might be of pathophysiological importance in conditions like myocardial ischemia or inflammatory disorders when LPA release is enhanced.


http://www.sciencedirect.com/science/article/B6WK6-4CTCV39-3/2/3a379b3db65a7b788b42df5d0f1e0552

Mutations of the KCNJ2 gene encoding the potassium channel Kir2.1 were previously shown to cause Andersen's syndrome (AS), a multisystem disease manifesting with developmental abnormalities, cardiac arrhythmias and periodic paralyses. We conducted a search for KCNJ2 mutations among 188 unrelated patients suspected to have long QT syndrome (LQTS). The screening was performed by denaturing high-performance liquid chromatography (dHPLC) and DNA sequencing. Two novel mutations of the KCNJ2 gene were detected: a missense threonine to alanine mutation (T75A) in the N-terminal region (family 1) and an in-frame deletion of two amino acids ([Delta]FQ163-164) in the M2 transmembrane region (family 2). In addition, a previously described silent polymorphism C1146T was detected. In family 1, some of the affected family members had a history of periodic muscle weakness characteristic of AS, but no dysmorphic features. The mean QTc interval of the affected members were 444 +/- 24 ms (family 1, n=7) and 456 +/- 8 ms (family 2, n=2). The mutations affect functionally important regions of the KCNJ2 channel protein: upon injection of the Xenopus oocytes with the wild type and mutant KCNJ2 constructs, the channel proteins were correctly synthesized and localized to the cell surface, but no measurable inward K+ current could be detected for the mutant KCNJ2 constructs. In conclusion, we report two novel loss-of-function mutations of the KCNJ2 channel, affecting different domains of the channel protein. Mutations of the KCNJ2 gene should be considered in genetic subclassification of LQTS patients, even in the absence of overt manifestations of AS.


http://www.sciencedirect.com/science/article/B6WK6-4F94YNH-4/2/77c721bb386528b4560ad87294814331

Under hypercontractile conditions associated with increased intracellular calcium, male hearts show enhanced ischemia/reperfusion injury compared to female hearts. Our aim in this study was to identify the specific estrogen receptor involved in this gender difference. Following brief treatment with isoproterenol, isolated mouse hearts were subjected to ischemia and reperfusion. Postischemic contractile function and infarct size were measured in wild-type (WT) male and female hearts, and female hearts lacking functional alpha estrogen receptor ([alpha]ERKO), or the beta estrogen receptor ([ss]ERKO). WT male hearts exhibited significantly less functional recovery and more necrosis than WT females. [alpha]ERKO female hearts exhibited ischemia/reperfusion injury similar to that observed in WT females, whereas [ss]ERKO females exhibited significantly less functional recovery than WT females and were similar to WT males. These data suggest that estrogen, through the [ss]-estrogen receptor, plays a role in the protection observed in the female heart. Furthermore, we identified genes that were differentially expressed in [ss]ERKO female hearts compared to [alpha]ERKO and WT female hearts, and found altered expression of a number of metabolism genes, which may be important in ischemic injury. We further showed that WT female hearts have increased ratio of carbohydrate to fatty acid metabolism relative to WT males.

http://www.sciencedirect.com/science/article/B6WK6-493P6N1-2/2/7ada2bfeccc1fc2cfe4bb2200739cd9

In response to vascular injury, adventitial fibroblasts can modulate their phenotype to myofibroblasts, cells that participate in arterial remodeling. However, the signaling mechanisms underlying the vascular myofibroblast differentiation remain unknown. Since protein kinase C (PKC) is a key enzyme for cell differentiation, we examined whether PKC isoforms were involved in the vascular myofibroblast differentiation. The association between PKC[alpha] and myofibroblast differentiation was investigated in cultured rat aortic fibroblasts treated with transforming growth factor-[beta]1 (TGF[beta]1). Confocal immunofluorescence microscopy indicated that fibroblasts expressed [alpha]-smooth muscle actin ([alpha]-SM actin) after TGF[beta]1 treatment. Moreover, TGF[beta]1 stimulation increased both PKC[alpha] mRNA expression (measured by real-time quantitative RT-PCR) and PKC activity (determined by histone-like pseudosubstrate phosphorylation) in adventitial fibroblasts. Western blot analysis indicated that PKC[alpha] protein expression was higher in TGF[beta]1-treated fibroblasts than in untreated cells. TGF[beta]1-induced expression of [alpha]-SM actin was inhibited in a dose-dependent manner by treating cells with a PKC inhibitor, calphostin C, and was abolished by depleting PKC[alpha] with antisense PKC[alpha] oligodeoxynucleotides. Our results demonstrate that TGF[beta]1 induces adventitial myofibroblast differentiation via a PKC[alpha]-dependent process.


http://www.sciencedirect.com/science/article/B6WK6-4BSF46K-X2/2/8770d569c5d38aad5bc2295ca89b99c5

We have searched for mutations in [alpha]-tropomyosin gene in 50 Japanese patients with hypertrophic cardiomyopathy (HCM) by means of polymerise chain reaction (PCR)-single strand conformation polymorphism (SSCP) analysis. Two missense mutations of the [alpha]-tropomyosin gene were detected in Japanese patients with familial HCM. Sequencing analysis revealed a C to T transition at codon 63 leading to a replacement of Ala with Val residue. and a G to A transition with replacement of Asp by Asn at codon 175. These missense mutations were found at residues which were markedly conserved across the species, and have been reported to interact with troponin T. This is the first report on a mutant [alpha]-tropomyosin gene in a Japanese Population. Familial HCM is a genetically heterogeneous disease in Japanese patients, similar to that reported in Caucasian kindreds.


http://www.sciencedirect.com/science/article/B6WK6-4BYRS5M-1/2/defba54e050b9e64e7204d6b22e11d
Transgenic mice with cardiac-specific over-expression of tumor necrosis factor-alpha (TNF1.6) progress to dilated heart failure. A significant inflammatory response precedes functional deterioration, and may contribute to cardiac damage in this model. To evaluate the underlying molecular mechanisms, we assessed the gene expression in six groups of mouse hearts defined by age, gender, and phenotype (n = 3/group) using Affymetrix microarray analysis. Phenotype was defined as compensated (in young TNF1.6) or decompensated (in older TNF1.6) via echocardiogram. Of the >1000 transcripts altered in the compensated hearts (fold change > 2, P < 50 genes were differentially regulated, including seven immunoglobulin genes. Real-time reverse transcriptase-polymerase chain reaction and cDNA microarray confirmed the Affymetrix data. Mac3+ macrophages, CD4+ T and CD45/B220+ B-cells were identified in both compensated and decompensated hearts. However, a large amount of IgG was found deposited in areas devoid of B-lymphocytes in the myocardium of decompensated TNF1.6 mice; no such accumulation was seen in the compensated or age-matched controls. Furthermore, nuclei density analyses showed a two-fold increase in the myocardium of both compensated and decompensated TNF1.6 mice (vs. WT). This study suggests that TNF-[alpha] over-expression activates not only the inflammatory response, but also humoral immune responses within the transgenic hearts. The autoimmune response occurs concomitantly with cardiac decompensation and may participate in triggering the transition to failure in TNF1.6 mice.


http://www.sciencedirect.com/science/article/B6WK6-4BJ0TXN-1/2/5ee3172d3cee0ac68ce3d81d381b8b38a

This study investigated the effect of fosinopril (Fos), valsartan (Val), and combination of both drugs (Fos + Val) on the cardiac and renal expression of aquaporin-1 (AQP1) and aquaporin-2 (AQP2) in congestive heart failure (CHF). A rat model of CHF was created by ligating the left anterior descending coronary artery to induce acute myocardial infarction (AMI). Rats were treated by Fos, Val, or Fos + Val for 4 weeks. In renal medulla and cortex, AMI was associated with 2.2- and 1.8-fold increase in AQP2 mRNA expression when compared with Sham-operated rats (medulla: 23.6 +/- 2.8 vs. 52.3 +/- 8.7%; P < 0.05 vs. Sham) and in the cortex (Fos, 21.2 +/- 6.7%; Val, 20.4 +/- 6.0%; Fos + Val, 18.9 +/- 7.5%; P = NS vs. Sham). Similarly, the AQP2 protein expression increased by 2.1-fold after CHF (P < 0.05). The renal and cardiac AQP1 gene and protein expressions were unaltered in CHF or by medical therapy. There was no observed cardiac AQP2 expression in all the study groups. Treatment with Fos, Val, or combination therapy was effective in preventing the upregulation of renal AQP2 gene and protein expressions in CHF rats caused by AMI.

Journal of Molecular Catalysis B: Enzymatic (4)


http://www.sciencedirect.com/science/article/B6TGN-4292K7N-
Screening of soil bacteria with allylbenzene resulted in a Bacillus megaterium strain, which hydroxylates simple hydrocarbons in high enantiomeric excess (ee up to 99%). Benzylic and nonbenzylic hydroxylation products were obtained, without the usually observed high preference for the benzylic position. The immobilization of the B. megaterium cells in alginate gel effectively improved the stability of the cells and increased the amounts of products formed, without loss of enantioselectivity. The product ratio ([alpha] vs. [beta] hydroxylation) was shifted towards benzylic hydroxylation, which suggests that at least two hydroxylating enzymes with distinct regioselectivity are involved. Comparison to free-cell fermentations in small- and large-scale bioreactors (up to 2000 ml) showed that the use of immobilized cells is advantageous, as they are easier to handle and yield higher amounts of oxidation products.


http://www.sciencedirect.com/science/article/B6TGN-498V7TP-1/2/704f4ac48b375df735434fcoe716cbe5c

Five catalytic and nine non-catalytic antibodies for insertion of a metal ion into porphyrin were generated by immunization with N-methyl mesoporphyrin (N-MMP) as hapten, which was designed to mimic the distortion of porphyrin toward a transition-state geometry in the reaction. In order to determine the features responsible for the catalytic activity, we characterized the properties of the catalytic and non-catalytic antibodies. The catalytic antibodies did not have higher affinity to N-MMP than the non-catalytic ones. All the antibodies, except one non-catalytic antibody, combined with ferric N-methyl mesoporphyrin (N-MMP-Fe) to form the respective antibody.N-MMP-Fe complex. The binding affinity of cyanide to ferric iron in the complexes agreed with that of free N-MMP-Fe, indicating that the protruding side of N-MMP-Fe in the complexes is exposed to solvents. All the complexes of the catalytic antibodies had a peroxidase-like activity, whereas those of the non-catalytic ones did not. This suggests that the metalation activity associates with the peroxidase-like one, so that there is a common residue acting as catalyst for both reactions. The amino acid sequence alignment shows that the catalytic antibodies contain a homologous heavy chain sequence in the third complementarity-determining region. Based on the results, the possibility that Asp(H96) in the region is the key residue responsible for the metalation and peroxidase-like activities is discussed.


http://www.sciencedirect.com/science/article/B6TGN-42BSCB2-B2/2/b1bf20ab6673cb58e9058ce0881a9ad2

Two hypothetical proteins of Escherichia coli, YbbQ and YhaE, show high sequence similarity to -threonine dehydrogenase. We cloned the genes encoding YbbQ and YhaE into E. coli JM109, and purified the expressed proteins to homogeneity from the E. coli clones. YbbQ consisted of two identical subunits with a molecular mass of 31 kDa, whereas YhaE was a homotetramer (native molecular mass, 124 kDa). Both enzymes required NAD+ as a coenzyme, and used serine as a substrate. -Serine was better substrate than -serine. YbbQ showed maximum activity at pH 11.0 for the oxidation of -serine, whereas the optimum pH of YhaE was 10.5. These enzymes also catalyzed the oxidation of glycerate and 3-hydroxyisobutyrate. The Vmax/Km
values of YbbQ for -serine, -serine, -glycerate, -glycerate, -3-hydroxyisobutyrate, and -3-
hydroxyisobutyrate were 1.22, 0.0054, 128, 4.97, 0.0295, and 0.718 [μmol min⁻¹ mg⁻¹ mM⁻¹],
and those of YhaE were 0.690, 0.057, 17.5, 0.650, 0.163, and 0.263 [μmol min⁻¹ mg⁻¹ mM⁻¹],
respectively. Thus, YbbQ and YhaE are NAD⁺-dependent dehydrogenases acting on 3-hydroxy
acids with 3-carbon chains, and -glycerate is the best substrate for both enzymes.

double mutants, Ser119Ala/Glu120Gly and Thr308Ala/Thr309Val, overcomes insoluble

http://www.sciencedirect.com/science/article/B6TGN-42RMNDX-2/2/3b4c9a4ce0defa43bac6f32e9b669f02

Of the isopenicillin N synthase isozymes, Streptomyces jumonjinensis isopenicillin N synthase
(sjIPNS) is the only insoluble isozyme produced in Escherichia coli during heterologous
expression. Hence, this study aims to optimise the soluble expression of sjIPNS in E. coli. By
lowering the cultivation temperature from 37 to 25[°C], previous studies have shown that the
solubility of IPNS from S. clavuligerus (scIPNS), S. lipmanii (slIPNS) and Nocardia lactamdurans
(nIPNS) in E. coli was much improved but the same could not be achieved for sjIPNS in this
study. To decipher the uniqueness of the elusive insoluble sjIPNS, its amino acid sequence was
compared to three other soluble isozymes, scIPNS, slIPNS and nIPNS. The computational
analyses revealed two positions with adjacent sites at 119/120 and 308/309, where by sjIPNS
differs significantly in terms of amino acid identities and properties. Site-directed mutagenesis
was then used to alter these sites to investigate their influence on solubility. The two double
mutants constructed, Ser119Ala/Glu120Gly and Thr308Ala/Thr309Val sjIPNS, were found to be
soluble at 28 and 25[°C], with optimal production up to 25.3 and 23.0%, respectively, of the
total soluble proteins. Furthermore, both mutants were found to be catalytically active. The results
of this study constitute the first report on the use of site-directed mutagenesis to successfully
transform the solubility of sjIPNS to closely resemble that of other soluble bacterial IPNS
isozymes.

Journal of Neuroscience Methods (10)

protein gene variant alleles by denaturing HPLC." Journal of Neuroscience Methods 139(2): 263.


Mutations in the human prion protein gene (PRNP) are responsible for hereditary diseases called
transmissible spongiform encephalopathies (TSE) and a polymorphic site at codon 129
determines sensitivity to infectious forms of these maladies. More recently, codon 129 has been
related to cognition performance in the elderly, in Alzheimer disease (AD) and in Down syndrome.
Furthermore, a rare polymorphism at codon 171 was described in 23% of patients with mesial
temporal lobe epilepsy related to hippocampal sclerosis (MTLE-HS), the most common form of
surgically remediable epileptic syndrome. Thus, a method that permits fast and efficient screening
of PRNP mutations and polymorphisms in patients, in high risk populations, and in family
members is desirable. In the present study, we established the conditions for analysis of the PRNP open reading frame using denaturing high-performance liquid chromatography (DHPLC), whereby unpurified PCR products were subjected to denaturing and reannealing steps leading to heteroduplex formation. We described specific profiles for the PRNP polymorphisms at codons 129 (M/V), 117 (A/A silent), 219 (E/K), 171 (N/S), and the octarepeat deletion using amplified DNA from 562 samples. The chromatograms for TSE-associated mutations at codons 102 (P/L), 183 (T/A), and 210 (V/I) were also determined. Specificity of the DHPLC profile for each PRNP variant allele was confirmed in 100% of the samples by direct and cloned DNA sequencing in addition to endonuclease digestion when applicable. Therefore, the present study shows that DHPLC is a rapid, highly accurate and efficient technique for the detection of PRNP genetic variants.


http://www.sciencedirect.com/science/article/B6T04-47RBBV9-4/2/54f9e0c8cc624e10309406ac6d9d3ffa

Nylon filter arrays spotted with differential display PCR (DD-PCR) clones and hybridized with radiolabeled cRNA generated from the source RNA pool (reverse Northern blot) provide a high-throughput means to screen clones for artifacts. Reverse Northern blots also confirm differential gene expression in parallel and require modest quantities of the source RNA pool. We describe a strategy to screen multiple candidates from DD-PCR by high-throughput ligation and transformation, followed by reverse Northern blotting. Purification of re-amplified DD-PCR clones and fabrication of nylon arrays was facilitated by a batch-processing protocol using the widely available Biomek(R) laboratory robot and Bioworks(TM) scripts (available from the authors). A strategy to screen out DD-PCR product artifacts of an inappropriate size was also employed. Using these approaches, we identified several mRNAs that are differentially expressed in response to venlafaxine, fluoxetine or desipramine antidepressant treatment in rat C6 glioma cell lines and are candidates for full length clone isolation using 5'-RACE. Such an approach provides a rapid means to eliminate the high percentage of false positive clones from DD-PCR and enables independent confirmation of differential gene expression patterns generated by various experimental conditions.


http://www.sciencedirect.com/science/article/B6T04-3SVS087-C/2/f32dd60265dec99686d7204d8e0eb2f5

A technique is described for determining the apolipoprotein E genotype (apo E; alleles [epsi]2, [epsi]3, or [epsi]4) from tissues which have been fixed with 4-10% formaldehyde and archived. The procedure requires efficient extraction and exhaustive purification of DNA from the fixed tissue. Because the fixation process renders the DNA largely crosslinked and/or sheared (therefore unsuitable for traditional analysis), a nested polymerase chain reaction (PCR) is employed (using two apo E gene specific primer pairs) to specifically amplify the polymorphic region of the gene. The genotype was then determined using previously reported HhaI polymorphisms that occur as a direct result of the variant codons responsible for the three alleles. This protocol permitted the successful genotyping of 90% (34 out of 38) of the archived brain samples from Alzheimer's disease (AD) patients. These samples included such extremes as a sample that had been stored for 12 years in formalin. This procedure permits the retrospective analysis of samples that had been processed and stored well before the original characterization
of apo E alleles as risk factors in AD. Finally, this approach is readily adapted to the analysis of any gene of interest, whether by restriction fragment length polymorphism or direct amplicon DNA sequencing. It is also a very robust assay for less stringent conditions such as DNA isolated from whole blood or frozen tissue.


http://www.sciencedirect.com/science/article/B6T04-4B42D2D-1/2/9b30501d8a2efcd11974171fbb66596f

The measurement of gene expressions in brains with neurodegenerative diseases is a major area of brain research. The objective of our research was to determine whether quantitative real-time PCR could measure messenger RNA (mRNA) expression in brains with post-mortem intervals beyond 12 h. In the present paper, we examined the quality of RNA from brain specimens of both Alzheimer's disease (AD) patients (n=13) and non-demented normal control subjects (n=6). To determine a unregulated endogenous reference gene in AD, we measured mRNA expressions of the commonly used reference genes [beta]-actin, 18S rRNA, and GAPDH. In addition, we determined whether post-mortem interval, brain weight, or age at death influences mRNA expression. Our real-time PCR analysis results indicate that mRNA expression can be detected in all brain specimens for [beta]-actin, 18S rRNA, GAPDH, and also synaptophysin, a known marker for AD. Further, using real-time PCR analysis, we found that [beta]-actin and 18S rRNA are differentially expressed in the brain specimens of both AD and control subjects, while GAPDH is similarly expressed in AD and control brain specimens. These findings suggest that GAPDH can be used as an endogenous reference gene in the study of AD brains. A comparative gene expression analysis also suggests that synaptophysin is down-regulated in AD brain specimens compared to control brain specimens.


http://www.sciencedirect.com/science/article/B6T04-3YJ9XNG-5/2/e8b8b4e03b0ffe1953a5ff20ad1450c3

We designed a rapid, simple and accurate PCR method to determine sexual identity of mouse fetuses collected on embryonic day 15. A multiplex PCR amplification was used to detect male-specific sequence (Sry) in DNA extracted from fetal livers through SDS denaturation followed by high salt extraction and precipitation. This extraction method resulted in sufficiently purified DNA in <1 h and was suitable for PCR. The DNA obtained was amplified using a robot thermal cycler for 33 cycles. The reaction was performed in 50 [mu]l, using two sets of primers specific for Sry gene (chromosome Y) and IL3 gene (chromosome 11). Amplification duration was 1.5 h. The assessment of the results was done by electrophoresis in 3% agarose run at high voltage. The 402 bp band (Sry) obtained identifies the male fetuses and the 544 bp product (IL3) confirms the correct amplification of the template DNA. The entire procedure took <4 h. The specificity of the method was confirmed by fluorescent in situ hybridization using a specific male probe on cultured male and female neural stem cells. This method allowed the preparation and culture of pure male and female neural stem cells from fetal tissue.

We developed a simplified protocol for sensitive quantitation of mRNA using polymerase chain reaction (PCR) amplification of cDNA made by reverse transcriptase (RT), as resolved with capillary electrophoresis (CE) and detected with laser-induced fluorescence (LIF). The conditions required for adequate accuracy of the simplified version of the RT/PCR quantitation, in which a single concentration of external standard and amplification to within or near the plateau phase are used, were established for assay of mRNAs expressed at high, moderate, and low abundance. The mRNAs for the cytosolic enzyme, glyceradehyde phosphate dehydrogenase (GAPDH) and the growth-associated protein GAP-43 in cultured SN49 neuroblastoma cells were used as target genes for high and moderate levels of expression, respectively. Using cultured mouse microglial cells (BV-2), we demonstrated the utility of this RT/PCR/CE/LIF protocol to quantitate a low-abundance mRNA, encoding a form of nitric oxide synthase (i-NOS) induced by treatment with endotoxin. The appearance of i-NOS mRNA after endotoxin treatment of BV-2 cells was confirmed by Northern blot analysis and in situ hybridization histochemistry, and functional enzyme activity was followed by release of nitric oxide (as nitrite) into the medium. The many advantages of the 'single-point' RT/PCR/CE/LIF protocol for quantitating mRNAs of interest include: simplified protocol, elimination of the use of radiotracers, high sensitivity and precision, and semi-automation of the quantitation phase of analysis.


Creutzfeldt-Jakob disease (CJD) and related disorders occur in sporadic, acquired and inherited forms. In sporadic, iatrogenic and new variant CJD the polymorphic codon 129 of the prion protein gene (PRNP) plays an important role for the susceptibility to the disease and for the clinical and neuropathological manifestations. All the inherited forms of CJD and related disorders are linked to point or insert mutations of PRNP. The analysis of PRNP is therefore important for a correct classification of these disorders and for the identification of novel mutations. The aim of the present study is to describe a fast and easy to perform method for the direct sequencing of the PCR amplified PRNP open reading frame, by using M13 tailed primers which allow a direct and rapid method of sequencing. The goodness of this method is demonstrated in the analysis of three sporadic CJD patients with different genotypes at codon 129 and three inherited cases bearing different point mutations of PRNP: the Pro102Leu mutation linked to Gerstmann-Straussler-Scheinker-syndrome, the Val210Ile mutation and a novel mutation at codon 211 (Gln211Glu) both associated to familial CJD.

We demonstrate that the degree of neuronal development of PC-12 cell differentiation can be quantified by the expression of neurofilament-L (NF-L) mRNA, when an optimal concentration of NGF (50 ng/ml) is used. During the first 7 days of NGF treatment, the relative amount of NF-L mRNA was found to increase continuously and to correlate with the outgrowth of neurites in a statistically significant way. Thus, mRNA expression is, under these conditions, a suitable means for reliably monitoring the differentiation of PC-12 cells as early as after 3 days of NGF treatment. The results obtained with 5 ng/ml NGF differ from those with 50 ng/ml: during the first 3 days of NGF treatment, neuronal outgrowth was less than with 50 ng/ml, although the NF-L mRNA levels did not depend significantly on NGF concentration. Beyond day 3, NF-L mRNA levels did not increase further at 5 ng/ml as opposed to 50 ng/ml NGF. These differences point to different signal transduction processes involved in neuronal differentiation at high and low NGF concentration. Expression of NF-L protein in response to NGF treatment was also demonstrated. In summary, our results stress that stable and sustained differentiation of PC-12 cells can only be achieved with 50 ng/ml NGF.


http://www.sciencedirect.com/science/article/B6T04-450HHKR-1/2/8b559299c7d239e59291a66e1e6b81c4

The common single nucleotide polymorphism at codon 129 of the prion protein gene is a key determinant of the genetic susceptibility to Creutzfeldt-Jakob disease (CJD). Recently, a molecular classification of sporadic CJD based on the M129V genotype in conjunction with other determinants was proposed. In the present study, we describe the development and evaluation of a rapid fluorescent-based assay to detect this polymorphism using the LightCycler system. The two polymorphic alleles could be clearly distinguished by their melting points at 52.1 and 60.4 [deg]C, representing the 129V and 129M alleles, respectively. These results were confirmed by DNA sequencing. We evaluated our test in 400 patient samples and found no deviations from the expected melting patterns. The calculated allele frequency for the M-allele was 0.66. Thus, we have established a rapid, reliable fluorescent assay for high-throughput detection of the prion protein M129V polymorphism.


http://www.sciencedirect.com/science/article/B6T04-4BNMPTG-6/2/6a31b351d53580bdc82cec1e3659e1f4

The enzyme 5[alpha]-reductase (5[alpha]-R) is present in many mammalian tissues, including the brain. The physiological importance of 5[alpha]-R in the brain derives from its capability to convert testosterone (T) to a more potent androgen, dihydrotestosterone (DHT), and to convert progesterone to its 5[alpha]-reduced derivative, precursors of allopregnanolone, potent allosteric modulator of the [gamma]-aminobutyric acid receptor (GABAA-R). 5[alpha]-R occurs as two isoforms, 5[alpha]-R type 1 (5[alpha]-R1) and 5[alpha]-R type 2 (5[alpha]-R2). We present an accurate, rapid, and modestly labor-intensive method to precisely quantitate 5[alpha]-R mRNA species in the cerebral cortex of the rat. This approach combines the high specificity of "one-step" reverse transcription-polymerase chain reaction (RT-PCR) with the sensitivity of laser-induced fluorescence capillary electrophoresis (LIF-CE). Both cDNA synthesis and PCR amplification are performed with the same enzyme and site-specific primers, improving the efficiency of cDNA synthesis. The specific target mRNA and a mimic DNA fragment, used as a competitive internal
standard, were co-amplified in a single reaction in which the same primers are used. The method presented in this paper enables a more efficient quantitative determination of 5[alpha]-R mRNA isozymes, and may lead to a better understanding of the role of 5[alpha]-R isozymes in the physiology of the central nervous system.

**Journal of Oral and Maxillofacial Surgery** (2)


http://www.sciencedirect.com/science/article/B6WKF-4CRR5T0-DM/2/8d77e1a217a1eb6cc10b2bb554627bdf

Purpose: Reactive arthritis (ReA) as a consequence of triggering Chlamydia trachomatis infections has been extensively studied to better understand inflammatory arthritis. This study investigated whether the presence of C trachomatis can be shown in the TMJ of patients with internal derangement.

Patients and Methods: Posterior bilaminar tissue removed from 31 patients (29 F, 2 M) during TMJ articular disc repositioning and posterior ligament repair was tested for the presence of C trachomatis. Cryosections were stained using a monoclonal antibody that identifies all chlamydial serovars. Highly specific polymerase chain reaction (PCR) assays independently targeting two genes of p p[C trachomatis also were performed; these assays also identify all serovars of this organism.

Results: TMJ tissue from 6 of 30 patients (20%) showed the presence of C trachomatis in the posterior bilaminar tissue on immunostaining. PCR screening identified 12 of 31 patients (39%) as having C trachomatis DNA in tissue, including four of six positive by immunostaining. All chlamydia-positive patients were female, with an average age of 36.7 years (15 to 48 years).

Conclusions: The presence of C trachomatis in the human TMJ has not been previously shown. The presence of this organism may serve as the pathogenetic mechanism for TMJ dysfunction, as demonstrated in other joints. Nonapparent chlamydial infection in females may also explain the marked prevalence of TMJ symptoms in women.


http://www.sciencedirect.com/science/article/B6WKF-4DW8KWB-M/2/fa8b7dc99ba7e3d23ab244ac144b7910

Purpose: This study was designed to determine whether multiple chondrocytes immersed in a new scaffold, 75:25 poly(L-lactide-[epsilon]-caprolactone) sponge coated with type I collagen (75-PLC scaffold), could be used to generate cartilage tissue in vivo and to evaluate the correlation between cartilage generation and the phenotype of the proliferated chondrocytes.

Materials and methods: Rat chondrocytes were suspended in 75-PLC scaffold at a density of 1 X 107 cells/mL after proliferation in a monolayer for 1 (P1) to 4 passages (P4) and implanted in nude mice for 4 weeks. Cells were characterized by the expression of genes encoding type II collagen, aggrecan, and type I collagen by Northern hybridization, and consequently, the newly formed tissue was evaluated histologically.

Results: The expression of aggrecan messenger RNA gradually decreased with the passaged cultures; however, the expression of type I collagen messenger RNA
increased with time. The cartilage formations in all specimens were found not only in P1 chondrocytes but also in P2 chondrocytes, although when P3 chondrocytes were grafted, approximately 50% of cartilage formation was still observed up to but not beyond P4. Conclusion It is suggested that cartilage tissue is generated with cultured chondrocytes up to P2 but not beyond P4. Northern blot analysis is useful for the assessment of whether the cells are capable of regeneration.

Journal of Orthopaedic Research (7)


http://www.sciencedirect.com/science/article/B6W7R-42M76BJ-S/2/6ed49f7dfc4b82d9560fd7a5bad1e968


http://www.sciencedirect.com/science/article/B6W7R-4CSYJG1-4/2/a23f6670fd8312bc5ded4fdb0daf3bc9

Overuse tendon injuries are common among elite and recreational athletes. Tendon healing may be enhanced at the cellular level through the use of exogenous growth factors; however, little is known about the endogenous expression of growth factors in healing tendon. This study describes the temporal expression of insulin-like growth factor-I (IGF-I), transforming growth factor-β (TGF-β), and collagen types I and III in healing tendon lesions. Collagenase-induced lesions were created in the tensile region of the flexor digitorum superficialis tendon of both forelimbs of 14 horses. Tendons were harvested from euthanatized horses 1, 2, 4, 8 or 24 weeks following injury. Gene expression was evaluated using Northern blot analysis (collagen types I and III), real time PCR (IGF-I and TGF-β), and in situ hybridization. Protein content was assayed by dye-binding assay (collagen types I and III), radioimmunoassay (IGF-I), ELISA (TGF-β), and immunohistochemistry. Samples were also processed for differential collagen typing, DNA and glycosaminoglycan content, and routine H&E staining. Microscopically, lesions progressed from an amorphous, acellular lesion soon after injury to scar tissue filled with collagen fibers and mature fibroblasts organized along lines of tension. Early lesions were characterized by immediate increases in expression of growth factors and collagen. Message levels for TGF-β peaked early in the wound healing process (1 week), while IGF-I peaked later (4 weeks), as the regenerative phase of healing was progressing. In the first 2 weeks after lesion induction, tissue levels of IGF-I protein actually decreased approximately 40% compared to normal tendon. By 4 weeks, these levels had exceeded those of normal tendon and remained elevated through 8 weeks. Message expression for collagen types I and III increased by 1 week following injury and remained elevated throughout the course of the study. Collagen type I represented the major type of collagen in healing tendon at all time points of the study. Based on these results, IGF-I, administered exogenously during the first 2 weeks following injury, may provide a therapeutic advantage by bolstering low endogenous tissue levels and enhancing the metabolic response of individual tendon fibroblasts.

[http://www.sciencedirect.com/science/article/B6W7R-45J8WMW-3/2/30e36d99e7e38b1fbf1b1a8187f8214d](http://www.sciencedirect.com/science/article/B6W7R-45J8WMW-3/2/30e36d99e7e38b1fbf1b1a8187f8214d)

Bone marrow contains many cellular elements that may contribute to fracture repair. We used a pluripotential stromal cell in a mouse model to demonstrate the presence of transplanted cells in fracture hematoma and subsequently in maturing fracture callus. Cells were transduced with traceable genes (lac Z and neomycin resistance) and traced in vivo after intravenous injection into syngeneic mice. These transduced cells home to bone marrow, suggesting that they might be detected in fracture callus. Cells were injected intravenously into mice and stabilized femoral shaft fractures were induced. Control mice received intravenous lactated-Ringer's solution prior to fracture. Callus tissue and marrow were examined histologically from 1 to 10 weeks after fracture to detect transplanted cells. Transplanted cells were detected in fracture callus in areas, and at times, of most active bone formation. Control specimens showed minimal staining of the callus tissue. Levels of the traceable gene in fracture callus increased, reached a peak between 3 and 4 weeks after fracture, then diminished and disappeared by 10 weeks post-fracture as woven bone at the fracture site was replaced by lamellar bone with cells from the host mouse. The results show that pluripotent bone marrow cells home to the marrow after systemic injection and localize in fracture callus.


[http://www.sciencedirect.com/science/article/B6W7R-45GWRRP-C/2/2e31743a46de1dfd7c95079dfdefa69d](http://www.sciencedirect.com/science/article/B6W7R-45GWRRP-C/2/2e31743a46de1dfd7c95079dfdefa69d)


[http://www.sciencedirect.com/science/article/B6W7R-44SJYB5-5/2/e0ba949f9c5fc6cd53c4369ac476c68e](http://www.sciencedirect.com/science/article/B6W7R-44SJYB5-5/2/e0ba949f9c5fc6cd53c4369ac476c68e)

Following vascularized bone autografts, osteocyte viability is largely maintained. Viable cells within a graft may be surviving graft-derived cells, their progeny, or host-derived cells from circulation or surrounding bone. This study was conducted to define the process of cell repopulation, within vascularized bone grafts. Using inbred Lewis rats, 30 female vascularized tibial bones were transplanted to syngeneic male recipients and 45 male grafts were transplanted to female recipients. Twenty-five female recipients were immunosuppressed with FK506 to prevent rejection caused by Y-chromosome related antigens. The grafts were assessed up to 24 weeks post-transplant by radiography, histology and semi-quantitative polymerase chain reaction (PCR) using both Y-chromosome and autosomal gene-specific primers. The female to male isograft transplants were useful to measure low levels of repopulation with host-derived cells, while male to female transplants more accurately quantified higher levels of cellular replacement. No host-derived cells were detected in the transplanted bone before six weeks. Thereafter, the ratio of host-derived cells gradually increased. By 24 weeks only 0.1-1.0% of graft-derived cells remained in the transplanted tibias. This study demonstrates that Y-chromosome-specific PCR is a useful tool to detect the cell lineage and cell repopulation following rat sex-mismatch.
vascularized bone grafting. Our results showed that donor cells in vascularized bone grafts were gradually repopulated with recipient cells. Correlation with histologic findings suggests that the periosteal hypertrophy observed by six weeks post-transplant results from graft-derived cells, while later remodeling is associated with host-derived cells.


http://www.sciencedirect.com/science/article/B6W7R-48TKCBN-1/2/8afc05807dbc3bb578c4cb63f2666c4d

Chimerism following allogeneic organ transplantation is a phenomenon known to occur and be associated with development of immunologic tolerance in allotransplantation. However, little is known about graft cell migration following vascularized bone allografting. In this study, chimerism was assessed following vascularized tibia transplantation from male DA or PVG donors to female PVG rat recipients using a semi-quantitative polymerase chain reaction for the Y-chromosome. FK-506 (Tacrolimus) was administered after transplantation for immunosuppression. All immunosuppressed PVG rat recipients of PVG bone grafts showed a high level of chimerism (1%) in the thymus, spleen, liver and cervical lymph nodes at 18 weeks post-transplant. Donor cells were also detected in the contralateral tibia and humerus. In non-immunosuppressed PVG rat recipients of DA bone grafts, donor cells were detected in the spleen in three of five rats within 2 weeks post-transplant. In these animals the bone grafts were severely rejected. In immunosuppressed PVG rat recipients of DA bone grafts, two of five, four of eight and eight of 10 rats showed low level chimerism (0.1%) in peripheral blood at 1, 12, and 18 weeks post-transplant. Six rats showed a high level of chimerism in the spleen and thymus. Histological studies revealed no rejection findings through 18 weeks post-transplant. Our results indicate that chimerism, or the presence of graft cells in host tissue, may occur in the face of acute rejection and be demonstrable following vascularized isograft and allograft living bone transplantation when chronic immunosuppression is maintained. Graft vascular patency during the short-term likely allows cellular migration, even in the face of acute rejection. Long-term survival and proliferation of graft marrow elements in host tissue may be possible with adequate immunosuppression.


http://www.sciencedirect.com/science/article/B6W7R-458P5XP-1/2/a2002819fec6975e9bdf5519d1dea600

Journal of Pediatric Surgery (14)

Background/Purpose: The matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) have been implicated in tumor invasion and metastasis. Net matrix degradation and proteolysis depend on the critical local balance between MMPs/TIMP-2. We attempted to determine their expression balance and to evaluate its importance with tumor progression.

Methods: Expressions of MMP-2, MMP-9, and TIMP-2 mRNAs was quantified by reverse-transcription polymerase chain reaction (RT PCR) in tumor tissues from 25 neuroblastoma patients.

Results: MMP-2, MMP-9, and TIMP-2 expression was observed in all the samples but with different trends. Increased expressions of MMP-2 mRNA was evident in advanced stages (Evans’ stage III and IV; \( P = .02; \) unpaired t-test), and in patients who died of progressive disease (\( P = .0001 \)). Whereas, the expressions of MMP-9 and TIMP-2 had no such significant association with clinical stages and prognosis. The ratio of MMP-2/TIMP-2 mRNAs was significantly higher in the advanced stages versus early stages (mean +/- SD = 1.66 +/- 0.65 and 1.11 +/- 0.34, respectively; \( P = .02 \)) and in patients who died of progressive disease versus alive patients (mean = 2.13 +/- 0.78 and 1.21 +/- 0.36, respectively; \( P = .0006 \)).

Conclusions: Coexpression of MMPs and TIMP-2 in neuroblastoma indicates the need to evaluate their expression balance. Significantly higher expression of MMP-2 mRNA and increased ratio of MMP-2 and TIMP-2 mRNAs in advanced stages or patients who have died of progressive disease suggests an association between elevated MMP-2 expression and poor prognosis. To establish the role of enzyme to inhibitor mRNA ratio as a reliable predictor, cohort studies with significant number of cases may be carried out.


Background/Purpose: The spontaneous mouse mutant Dominant megacolon (Dom) represents the model of the Waardenburg-Hirschsprung's disease, a syndromic pathology, characterized by the association of pigmentation defects (PD), deafness, and Hirschsprung's disease (HD). The defect in Dom mouse is caused by a spontaneous mutation of the gene encoding the Sry-related transcription factor Sox10. This mutation affects several aspects of neural crest development leading to combined enteric innervation and pigmentation defects, both in mouse and human. The purpose of this report is to define, by enzymo-histochemical techniques routinely used for the diagnosis of human Hirschsprung's disease (AChE, LDH, NADPH-diaphorase), the innervative patterns of the affected gut.

Methods: Fifty-four siblings of Heterozygous Dom/+ mice underwent autopsy and were genotyped by direct sequencing of polymerase chain reaction (PCR) products for Sox10 mutations. The enteric nervous system of all the mice was studied by histochemical techniques indicated above. Results: Genotyping showed that 43 mice were Dom/+ and 11 were Wild type +/+ . Wild-type +/+ mice were used as control. The correspondence between genotype and at least 1 phenotypic aspect (PD or dysganglionosis) was present in 93% of cases (41 of 43). Among the Dom/+ mice, dysganglionosis was present in 79% of cases and PD in 90% of cases. Moreover, among Dom/+ mice, excluding those whose mantle was not evaluated as dead just after birth, PD and dysganglionosis (complete phenotype) were present in 68% of cases. Among the Dom/+ mice, dysganglionosis was present in 79% of cases and PD in 90% of cases. Moreover, among Dom/+ mice, excluding those whose mantle was not evaluated as dead just after birth, PD and dysganglionosis (complete phenotype) were present in 68% of cases.

Conclusions: The histochemical methods that we used proved to be useful for identification of different aganglionic (AG), hypoganglionic (HG), and normoganglionic segments of Dom/+ mouse gut studied in longitudinal sections. Unlike humans, control mice (Wild type +/+ ) presented a rich component of AChE nerve fibers, whereas Dom/+ mice with dysganglionosis presented a decrease in AChE-positive nerve fibers. These data confirm the variable phenotypic penetrance in heterozygous mice. Because dysganglionosis in this animal model (Dom/+ ) was evident in 79% of cases (AG or HG), we concluded that Dom mice could represent important models for
further experimental studies.


http://www.sciencedirect.com/science/article/B6WKP-4CTD5NV-H/2/d8274c1e8cd959a3547d2e14388ed30

Background/Purpose: Congenital cystic adenomatoid malformations (CCAM) are lung lesions that demonstrate abnormalities of both mesenchymal and epithelial tissues. The pathogenesis of these tumors remains unknown. Because normal organogenesis requires a balance between cell proliferation and programmed cell death (apoptosis), the authors hypothesized that CCAM results from an increase in cell proliferation or a decrease in apoptosis within the developing lung, possibly mediated by keratinocyte growth factor (KGF). Methods: To examine cell cycle control in CCAM, we measured indices of cell proliferation and apoptosis in lesions requiring fetal (n = 4) or neonatal (n = 8) resection compared with those of normal fetal (14 to 28 week's gestation; N = 14) and neonatal (n = 3) human lung. Cell proliferation was analyzed by immunostaining for a proliferation marker (Ki-67). apoptosis was examined using an in situ digoxigenin end-labeling technique to localize apoptotic bodies. The expression of KGF protein and KGF mRNA in CCAM and normal lung was examined using immunohistochemistry and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR).

Results: CCAM lesions in general showed a twofold increase in cell proliferation index (19.2% +/- 1.4% v 9.6% +/- 0.7%, P v 4.5 +/- 0.5, P

Conclusions: These results demonstrate that CCAM differs from normal lung by increased cell proliferation and decreased apoptosis. The increased proliferation does not appear to be mediated by the pneumocyte mitogen KGF. An examination of factors that control cell proliferation and apoptosis in CCAM may provide further insight into the pathogenesis of this tumor.


http://www.sciencedirect.com/science/article/B6WKP-4CR8G4W-X/2/7e3c796839beca6b803d51f783f27a5c

Nitric oxide has been described as an inhibitory neurotransmitter to mediate smooth muscle relaxation in the mammalian gastrointestinal tract. The enzyme neuronal nitric oxide synthase (NOS) catalyzes the formation of NO. The authors examined the expression of neuronal NOS gene at the mRNA level in intestinal specimens from seven patients who had Hirschsprung's disease (HD) using reverse transcription polymerase chain reaction (RT-PCR) technique. With 35 cycles of PCR reaction, substantial signals of neuronal NOS mRNA were observed in the ganglionic bowel in all seven patients, whereas in the aganglionic bowel, neuronal NOS signals were weak in three patients, and undetectable in four patients. By increasing the PCR cycle to 40 cycles, barely detectable signals were observed in two of the latter four patients. Semiquantitative analysis in the three patients who showed weak signals with 35 cycles of PCR indicated that neuronal NOS mRNA expression in aganglionic bowel was decreased at least 1/50 to 1/100 of the level of expressed in ganglionic bowel. Because absence or low level of expression of neuronal NOS mRNA may lead to impaired production of NO, our observations suggest that motility dysfunction in HD may be as a result of markedly decreased or no expression of the neuronal NOS gene at the mRNA level.

http://www.sciencedirect.com/science/article/B6WKP-4CT1124-6R/2/331f56b7618ff14ad8823b948f30a7f2

The RET proto-oncogene is a major cause of Hirschsprung's disease (HD) as demonstrated by the experimentally produced intestinal aganglionosis in mice with a null mutation of this gene and by the increased evidence of RET mutations in patients with HD. To evaluate the possible implication of the RET gene for the development of HD, we examined mRNA expression level of the RET gene in the bowel specimen of seven HD patients by using the reverse transcription-polymerase chain reaction technique. A significantly less intense signal for RET mRNA was found in the aganglionic bowel compared with the ganglionic bowel. In the hypoganglionic transitional zone, the RET mRNA level was higher than that of an aganglionic segment but lower than that observed in the ganglionic portion. In two patients where semiquantitative analysis was performed, the RET mRNA level in the aganglionic bowels was estimated to be approximately 1/500 of that in the ganglionic bowels. Because expression of RET mRNA plays an important role in establishing the enteric neuronal lineage, decreased RET mRNA expression in the aganglionic bowel may suggest maldevelopment of neural crest-derived cells in Hirschsprung's disease.


http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-DD/2/50b4b8c92b19029c9cc8d57dfe86d657

Background: During lung development, platelet-derived growth factor-BB (PDGF-BB) is maximal during the canalicular stage and decreases by the saccular stage. PDGF-BB stimulates lung growth by increasing cell proliferation. Fetal CCAMs have been shown to have an elevated proliferative index, but it is not known why some CCAMs rapidly enlarge in utero and cause fetal hydrops. The authors hypothesized that the high proliferative index and rapid enlargement of some fetal CCAMs may be caused by persistently elevated PDGF-BB production compared with normal fetal lung. Methods: To test this hypothesis, tissue was obtained at the time of resection from two fetal CCAMs (22 weeks), three full-term CCAMs, and three normal fetal lungs (21 to 22 weeks). PDGF-BB production by fetal CCAMs was compared with normal age-matched fetal lung using immunohistochemistry, reverse transcriptionase-polymerase chain reaction (RT-PCR), and Western blot analysis. Results: CCAMs resulting in fetal hydrops and requiring fetal resection had strong mesenchymal immunostaining for PDGF-BB next to epithelial lined cysts, increased PDGF-B gene expression by RT-PCR, and elevated PDGF-BB protein by Western blot, compared with normal age-matched fetal lung. Term CCAMs had minimal PDGF-BB staining, PDGF-B gene expression, and PDGF-BB protein production. Conclusions: CCAMs that grew rapidly and progressed to hydrops, requiring in utero resection, demonstrated increased mesenchymal PDGF-B gene expression and PDGF-BB protein production compared with age-matched normal fetal lung, which may, in part, be responsible for the autonomous growth and proliferation seen in hydropic fetal CCAMs.

Background/Purpose: Wilms' tumor is the most common renal malignancy of childhood. Loss of heterozygosity (LOH) at 16q is seen in about 17% of cases and has been associated with a poor prognosis. To more precisely define the pattern of 16q deletion exhibited by Wilms' tumor, the authors performed a detailed LOH analysis of 96 specimens using polymorphic microsatellite repeat markers. The authors also evaluated the neoplasms for the presence of microsatellite instability (MSI). Methods: A total of 96 DNA samples were studied using polymerase chain reaction-based LOH analyses amplifying polymorphic microsatellite repeat markers. Screening for MSI using 2 additional genetic markers also was carried out. Results: The authors found 16q LOH in 14 of the specimens evaluated. Comprehensive analysis of these LOH-positive specimens showed a region of loss spanning 16p11.2-q22.1 and a separate distal region of LOH at 16q23.2-24.2. The distal region of deletion is very small, estimated to be approximately 2.4 megabases. In addition to the observed LOH, 2 specimens were found to consistently exhibit MSI, which has not been reported previously in Wilms' tumor. Conclusions: The smallest consensus region of deletion in our analysis of Wilms' tumor 16q LOH measures 2.4 megabases at 16q23.2-q24.2. Additionally, MSI was present in a subset of tumor specimens suggesting that defects in DNA mismatch repair may contribute to the pathogenesis of Wilms' tumor. J Pediatr Surg 35:891-897. Copyright (c) 2000 by W.B. Saunders Company.


Background: Recently, the endothelin-3 (EDN3) and endothelin-B receptor (ENDRB) gene have been recognized as susceptibility genes for Hirschsprung's disease (HD). However, gene mutations have been observed only in limited cases, and the role of EDN3 in the pathogenesis and motility dysfunction in HD is not understood fully. To evaluate the possible implication of EDN3 and EDNRB for the development of HD, we examined the EDN3 and EDNRB mRNA level in bowel specimens of HD patients. Methods: Entire resected specimens of colon were obtained from 14 patients with HD. Eight age-matched control patients without gastroenteric disorders also were examined. mRNA was extracted from ganglionic and aganglionic segments of the HD specimens and normal colons. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to evaluate the relative amount of EDN3 and EDNRB mRNA. Results: In normal colon, constant EDN3 and EDNRB mRNA expression was observed. In HD, EDN3 and EDNRB mRNA expression was observed. In HD, EDN3 and EDNRB mRNA levels were decreased both in ganglionic and aganglionic segment in 2 cases. In 6 cases, EDN3 mRNA expression was decreased in aganglionic segment and in another 2 cases, EDNRB mRNA expression was decreased in aganglionic segment. In the remaining 4 cases, EDN3 and EDNRB mRNA levels were similar to controls. Conclusion: The authors' findings indicate that loss of EDN3 and EDNRB function may be involved in the maldevelopment of neural crest-derived cells causing HD in many patients.

Purpose: The aim of this study was to investigate the expression and distribution of SK2 and SK3 channels in the normal and Hirschsprung's disease (HD) bowel. Methods: Full-thickness colonic specimens were collected at pull-through operation from 10 patients with HD and from 6 patients during bladder augmentation. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for SK2 and SK3 channels and double immunostaining using anti SK2/c-kit, SK3/c-kit, SK2/\alpha-SMA, and SK2/PGP 9.5 antibodies was performed. Immunolocalization was detected using laser scanning microscopy. Results: RT-PCR analysis showed strong expression of SK2 and SK3 mRNA in the normal human bowel and significantly reduced SK3 expression in the aganglionic bowel (P Conclusion: The results of this study provide the first evidence for the presence of SK2 and SK3 channels and for the immunocolocalization of SK3 channels in the ICCs in the normal human colon. Decreased expression SK3 channels in the aganglionic bowel may contribute to motility dysfunction in HD.


http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-CS/2/611882acee24d34f5c15132498ef7902

Background/Purpose: Fetal tracheal occlusion (TO) accelerates lung growth in normal and hypoplastic fetal lung. The mechanism of accelerated lung growth remains unknown but may be a result of growth factor induction. Previous studies of growth factors induced by tracheal ligation have characterized mRNA rather than protein expression. Although the transforming growth factor-[beta] (TGF-[beta]) family participates in normal lung morphogenesis, its role in lung growth after TO is unclear. The authors hypothesize that TGF-[beta] expression is increased with TO and may contribute to the accelerated lung growth seen after TO. Methods: Diaphragmatic hernia (DH) was created in 80-day-gestation sheep (n = 6; term, 145) by excising the left diaphragm. At 110 days, the trachea was occluded (n = 4) with a clip. DH controls (n = 2) were not occluded. Fetuses were killed at 139 days, and lung samples were snap frozen for tissue analysis. Non-DH control lungs were harvested from full-term animals (n = 2). TGF-[beta] mRNA was analyzed by semiquantitative reverse transcriptionase-polymerase chain reaction (RT-PCR). TGF-[beta] protein was assessed by Western blot analysis. Results: TGF-[beta]1 mRNA and protein were not increased with tracheal ligation compared with either non-DH or DH controls. TGF-[beta]2, however, was markedly increased, at both the mRNA and protein level, in ligated lungs compared with nonligated controls. Conclusions: TGF-[beta]2 protein, but not TGF-[beta]1, is increased in the hypoplastic lungs of fetal sheep after tracheal occlusion. Increased TGF-[beta]2 expression appears to result from increased or prolonged expression of mRNA transcripts. This is the first study to document a change in growth factor protein levels after TO. Increased TGF-[beta]2 expression may contribute to accelerated lung growth and decreased surfactant production observed after tracheal occlusion.


http://www.sciencedirect.com/science/article/B6WKP-4FH06RR-B/2/c0d35520406291e8273e9e9ab76de649

Purpose: The aim of this study was to examine the association between E-cadherin expression and markers of Wilms' tumor aggression, including metastasis and recurrence. Methods: Forty Wilms' tumor samples from the National Wilms' Tumor Study Group underwent immunohistochemical staining for E-cadherin. Tumor stage at diagnosis, recurrence, and loss of heterozygosity at 16q status was known for each of the tumor samples. E-Cadherin cell staining
was defined as high (>33%) or low (ResultsWilms' tumors presenting with metastatic (stage IV) disease demonstrated decreased levels of E-cadherin expression compared with localized tumors (stage I) (Fisher's Exact test, P ConclusionIn this study, the authors have found an association between decreased E-cadherin expression and metastatic Wilms' tumor. Mutations identified may help identify a mechanism for downregulation. The functional significance of these mutations is supported by the conserved nature of the amino acids across multiple species. The authors believe these findings support the involvement of E-cadherin in the evolution of Wilms' tumor.


http://www.sciencedirect.com/science/article/B6WKP-4BT1J8H-B/2/5de3502302e1d8fa581162ff20e39c65

Background/purpose The renin-angiotensin system plays an important role in pulmonary artery remodelling. Several polymorphisms of genes encoding for components of the renin angiotensin system such as the angiotensin converting enzyme (ACE), the angiotensinogen (AGT) gene, and the angiotensin II type 1 receptor (AT1R) have been associated with the development of pulmonary hypertension. The aim of this study was to investigate the ACE I/D genotype, the M235 T polymorphism of the AGT gene and the A1166 C polymorphism of AT1R in the lungs of congenital diaphragmatic hernia (CDH) complicated by persistent pulmonary hypertension (PPH) in the newborn. Methods Genomic DNA was extracted from archival paraffin-embedded lung tissue from 13 newborns with CDH complicated by PPH and from 9 controls. Genotyping for the I/D-ACE, the M235 T-AGT, and the A1166 C-ATIR gene polymorphisms were determined by a polymerase chain reaction-based method with appropriate restriction digest when required. Results In controls, ACE genotype distribution of DD, ID, and II was 11%, 33%, and 55%, respectively, whereas in CDH it was 70%, 15%, and 15%, respectively. The ACE-DD genotype was significantly higher in CDH compared with controls (P Conclusion These data suggest that D allele of the ACE gene insertion/deletion polymorphism and angiotensinogen M235 T polymorphism may be associated with PPH in newborns with congenital diaphragmatic hernia.


http://www.sciencedirect.com/science/article/B6WKP-4C2PRK5-6/2/3b85b36f672971d5520dfa80524af88

Background Although the pathogenesis of esophageal atresia with tracheoesophageal fistula (EA/TEF) remains unknown, it has been shown that despite its esophageal appearance, the fistula tract originates from respiratory epithelium. The authors now hypothesize that defects in fibroblast growth factor (FGF) signaling contribute to the esophaguslike phenotype of the fistula tract. FGF2R is critical to normal lung morphogenesis and occurs in 2 isoforms (FGF2R1IIib and FGF2R1IIc), each with different ligand-binding specificity. To characterize FGF signaling in the developing EA/TEF, the authors analyzed levels of FGF2R splice variants in experimental EA/TEF. Methods The standard Adriamycin-induced EA/TEF model in rats was used. Individual foregut components from Adriamycin-treated and control embryos were processed for real-time, fluorescence-activated semiquantitative reverse transcriptase polymerase chain reaction on gestational days 12.5 and 13.5. Results Both fistula tract and Adriamycin-treated or normal esophagus showed significantly lower levels of FGF2R1IIib than either Adriamycin-treated lung buds (E12.5, P =.02; E13.5, P P P P P Conclusion Levels of FGF2R in the developing fistula
tract resemble that of distal esophagus rather than developing lung. This defect in FGF2RIIib signaling may account for the nonbranching, esophagus-like phenotype of the fistula, despite its respiratory origin.


http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-CW/2/97913541194d61856d52a8c7fafa9c794

Background/Purpose: Collagen and elastin, the predominant components of the lung connective tissue network, have been suggested to have an important influence on lung compliance and maximal expansion. Decrease in lung compliance and distensibility often is seen in human congenital diaphragmatic hernia (CDH) lung as well as in experimentally produced CDH lung. The aim of this study was to investigate mRNA levels of tropoelastin and [alpha]1 (I) procollagen, the precursors of elastin, and type I collagen, respectively, in CDH lung and to determine whether antenatal dexamethasone treatment has any effect on the production of these extracellular matrix proteins.

Methods: CDH model was induced in pregnant rats after administration of 100 mg nitrofen on day 9.5 of gestation (term, 22 days). Dexamethasone (0.25 mg/kg) was given on day 18.5 and 19.5. Cesarean section was performed on day 21. The fetuses were divided into three groups: group I, normal controls; group II, nitrofen-induced CDH; and group III, nitrofen-induced CDH with antenatal dexamethasone treatment. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to evaluate relative amounts of tropoelastin and [alpha]1 (I) procollagen mRNA.

Results: Levels of both tropoelastin and [alpha]1 (I) procollagen mRNA were significantly increased in group II compared with group I (P Conclusions: The increased local synthesis of tropoelastin and type I procollagen in CDH lung may be responsible for the increased rigidity and decreased compliance observed in the CDH hypoplastic lung. Glucocorticoids have no effect on pulmonary tropoelastin and [alpha]1 (I) procollagen gene expression in CDH lungs.


http://www.sciencedirect.com/science/article/B6TGX-3W0FSK8-4R/2/d8aa7b79ec12e5c82f780aabd6f9d66a

Detection of point mutations in genomic DNA is important for diagnosis of inherited characteristics and genetic diseases. A point mutation in a specific region of DNA amplified by polymerase chain reaction (PCR) can be detected with single-strand conformation polymorphism (SSCP) analysis. Analysis of SSCP by laser-induced fluorescence capillary electrophoresis in entangled polymer solution (CE-LIF) has been developed in the present paper. K-ras genes including seven mutations were amplified with primer labeled with Texas Red at its 5’ end. The labeled PCR products were dissociated to single strands by heating and separated with capillary gel electrophoresis and He-Ne laser-excited fluorescence detection. Our results suggest that all fragments having normal (Gly) and mutated (Ala, Arg, Cys, Ser, Val, Asp) sequences at codon 12
can be distinguished. Analysis of SSCP with CE-LIF is well suited for clinical analysis of SSCP because of its high sensitivity, resolution, reproducibility and speed.


http://www.sciencedirect.com/science/article/B6TGX-45JYJC6- F/2/0e1e018a4a32672b457a910209872898

In a previous paper, optimal reaction conditions were determined for the RT-PCR part of a quantitative enterovirus specific RT-PCR ELISA method (J. Pharm. Biomed. Anal., 25 (2001) 131-142). In order to obtain a detection limit as low as possible, the ELISA part of the procedure was optimised as well. This was done by investigating the influence of seven factors at three levels in a multivariate approach. A reflected two-level screening design, derived from a Plackett-Burman design, was used. Optimal reaction conditions were established by calculation and by evaluation of the effects of the factors on the measured absorbance of the ELISA detection. Under these conditions, the linear range and detection limit of the test were determined and compared with the ELISA conditions before optimisation. The optimised RT-PCR ELISA will be used to study a possible longitudinal relationship between enteroviruses and the development of multiple sclerosis and juvenile diabetes.


http://www.sciencedirect.com/science/article/B6TGX-42M7828- J/2/6637db87972d5e7573113fb04e43c931

In order to obtain a detection limit as low as possible for a quantitative enterovirus specific RT-PCR ELISA assay, optimal reaction conditions, which give rise to the highest response, need to be determined. This was done by investigating the influence of 13 factors, selected from RT and PCR, in a multivariate approach by means of a well-balanced three-level screening design, derived from a three-level Plackett-Burman design. Optimal reaction conditions could be determined by calculation and evaluation of the effects of the different factors on the response, i.e. the measured absorbance of the ELISA detection. The method will be used to study a possible longitudinal relationship between enteroviruses and the development of multiple sclerosis and juvenile diabetes.


http://www.sciencedirect.com/science/article/B6TGX-492098W- 3/2/ea0a9c5bb172d82c9c65c6cad3e056f7

Quantitative reverse transcription-polymerase chain reaction enzyme linked immunosorbent assay (RT-PCR ELISA) is the method of choice to study positive- and negative strand viral RNA synthesis during poliovirus replication. In comparison with other methods used for this purpose, it
fulfils all necessary requirements to accurately determine RNA of different polarity. It combines high specificity, high sensitivity, safety, speed, and the ability to perform quantitative analysis. The enterovirus specific RT-PCR ELISA method described in this work, was used to determine quantitatively the amount of de novo poliovirus positive- and negative strand RNA synthesis at different time-points in the viral replication cycle, both in presence and absence of the viral RNA synthesis inhibitor guanidine hydrochloride.


Quantification of virus-like RNA sequences in biological fluids, like serum and cerebrospinal fluid, requires an RNA extraction method that is both reproducible and fast. Three RNA extraction methods were tested on enteroviruses: (1) the acid guanidine thiocyanate-phenol/chloroform (AGPC) method; (2) a method based on differential precipitation of the RNA and (3) a ‘bind-wash-elute’ system based on silica-gel membrane binding. The latter two methods yielded a comparable detection limit as measured by RT-PCR ELISA. The detection limit for the AGPC method was 10 times higher. The relative standard deviation for the bind-wash-elute method (3%) was superior to that of the other methods tested (both 20%) and provides a reliable and fast method to extract (viral) RNA from biological fluids for quantification by RT-PCR.


Studies were conducted to characterize assays for the isolation and quantitation of rat cytochrome P450 (CYP) 3A isoforms from hepatic and intestinal tissues. Isolated intestinal microsomes were analyzed for their alkaline phosphatase activity and CYP 3A immunoreactivity. The involvement of CYP 3A in the in vitro hydroxylation of midazolam (MDZ) was also evaluated using isoform specific chemical and antibody inhibitors. The effect of glycerol (a common constituent of the microsomal reconstitution buffer) concentration on in vitro MDZ hydroxylation was also investigated. Additionally, to verify that the intestinal preparation was adequate for use in studies investigating the induction of CYP3A at the MRNA, protein, and catalytic activity within a single animal, a separate induction study was carried out with the CYP 3A inducer dexamethasone (DEX). A reverse transcription-polymerase chain reaction (RT-PCR) assay and a quantitative Western blotting method were used to reliably detect differences in CYP 3A mRNA and immunoreactivity between DEX- and vehicle (VH)-treated tissues. The in vitro hydroxylation of MDZ evaluated CYP 3A catalytic activity and identified increases in CYP 3A activity caused by DEX in comparison to VH. Collectively, these described techniques provide an experimental model to study xenobiotic induction of rat hepatic and intestinal CYP 3A from the molecular to the
The cDNAs encoding both A and B subtypes of the human endothelin receptor have been inserted into mammalian cell expression vectors that utilize the human globin gene, locus control region. These constructs have been introduced into murine erythroleukemia cells and inducible high level expression of the receptors has been achieved (~1.5-pM/mg membrane protein and ~13,500 binding sites/cell for both receptor subtypes). Cell lines expressing these receptors were obtained on a rapid time scale (3-4 weeks), facilitated by the need for the analysis of only small numbers of cell clones/receptor (~6). Competitive binding assays with endothelin-1 gave IC50s of 130 +/- 30 pM for endothelin-A receptor and 160 +/- 30 pM for endothelin-B receptor. Similar studies with the different isoforms of endothelin, sarafotoxin-S6b and -S6c, BQ123 and BQ3020, all gave the expected selectivity profiles. The IC50s for all compounds were in close agreement with those reported for native receptors. Thus, this expression system, which has several advantages over other described expression systems, is capable of rapidly providing large quantities of receptor for detailed pharmacological analyses or drug screening. In addition, the expressed receptors display the expected pharmacological profiles in the absence of any complicating, competing interactions from other subtypes or binding sites.


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http://www.sciencedirect.com/science/article/B6TH0-44K8208-5/2/9b362dbc93a487920381ee71cb3d2d35

The effects of the reaction photosensitized by 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT) on a mouse lymphoma cell line have been examined. Using the hypoxanthine phosphoribosyltransferase (HPRT) locus as target gene, a mutagenic effect of the photoreaction can be detected concomitantly with a loss of cell viability. Isolation of HPRT deficient clones has permitted a molecular characterization of the mutational pattern induced by the photosensitization reaction mediated by HMT. Southern blotting analysis demonstrated that the HPRT deficiency could not be correlated with gene deletions larger than 300 bp. Using polymerase chain reaction on both DNA and cDNA, amplification products have been cloned into M13mp18 and sequenced. Base transversions targeted on thymine residues have been located in exon 2, 3, 8 and 9 together with spontaneous frameshift mutations occurring in a run of guanine residues in exon 3. HPRT deficiencies owing to mutations arising in the HPRT promoter region have also been observed. Dot and Northern blot analysis revealed that the photoreaction could lead to either a
reduced level of gene transcription or to a complete absence of HPRT m-RNA. Using polymerase chain reaction (PCR) amplification and agarose gel electrophoresis, deletions in the HPRT promoter have been observed and correlated to deficient enzyme expression.

Journal of Physiology-Paris (1)


http://www.sciencedirect.com/science/article/B6VMC-46GC3BR-2B/2/fb1e7f6d2294cd07a650114567a95585

Background and aim: A group of the proinflammatory and chemotactic cytokines (chemokines) has been considered as an important factor in the pathomechanism of different bacterial diseases, among them the common Helicobacter pylori infection. Experimental results obtained with gastric biopsy samples of H. pylori positive patients, and with H. pylori infected tumor originated gastric cell lines indicated that these cytokines have essential roles in the development and maintenance of the immune response and inflammation of the gastric mucosa during H. pylori infection. Although the mRNA expression was shown in these biopsy samples and cell lines, it is not yet proved that the normal gastric mucosal epithelial cells themselves express these cytokines. The establishment of a gastric surface mucous cell line with non-tumor origin (GSM06), and the usage of Helicobacter felis as a model of the classic H. pylori infection gave us the possibility to check this question. Materials and methods: in this study GSM06 cells were infected with different numbers (105, 106, 107, 108, 109 bacterium/ml medium) of H. felis for two different time periods (2, 4 h). Cells treated with medium only were used as control. Then the mRNA expression of the following cytokines was measured by RT-PCR method in the GSM06 cells: proinflammatory cytokine IL1-beta, and chemokine RANTES, eotaxin, MCP-1, MIP1-alpha and MIP1-beta. Results: we found that neither mRNA of the investigated cytokines was expressed constitutively, however the GSM06 cells expressed the mRNA of each cytokine during H. felis infection. Conclusion: our results prove that normal gastric surface mucous epithelial cells express immunologically active peptides during H. felis infection. We may suppose that the epithelial cells of the gastric mucosa contribute to the immune response and inflammation by expressing proinflammatory (IL1-beta) and chemotactic (RANTES, eotaxin, MCP-1, MIP1-alpha and beta) cytokines during H. pylori infection in human.

Journal of Plant Physiology (1)


http://www.sciencedirect.com/science/article/B7GJ7-4FDJ73D-3/2/3c64f58eabfda0d5c4df315c914ef08d
Summary

When tomato was grown in either "Breinigerberg" soil, which has a high content of Zn and of other heavy metals or in non-polluted soil enriched with up to 1 mM CdCl₂, plants colonized with the arbuscular mycorrhizal fungus (AMF) Glomus intraradices grew distinctly better than non-mycorrhizal controls. An analysis of differential mRNA transcript formations was performed on several plant genes coding for products potentially involved in heavy metal tolerance. Northern blot analyses indicated that the mRNA from either roots or leaves was not differentially expressed in the case of LePCS1 (coding for phytochelatin synthase), Lemt1, Lemt3 and Lemt4 (for metallothioneins) or LeNramp2 (for a broad range heavy metal transporter) in both mycorrhizal and non-mycorrhizal plants, grown either with or without heavy metals. In contrast, Lemt2 was strongly expressed only in non-AMF-colonized roots, and only after growth in the Breinigerberg soil or in the presence of high CdCl₂-concentrations. AMF colonization distinctly reduced the level of Lemt2 transcripts. This was also the case for the root specific LeNramp1 transporter, however, only after growth in the Breinigerberg soil, but not under Cd-stress.

Likewise, the levels of LeNramp3 transcripts were reduced by the AMF colonization in roots, but not in leaves. Quantitative Real-Time RT-PCR-experiments performed with Lemt2, LeNramp1 and LeNramp3 largely corroborated the Northern analysis data. In situ hybridization experiments with Lemt2 and LeNramp1 showed that both genes were strongly expressed throughout the plant cells in non-colonized roots, whereas colonized roots revealed only few signals restricted to some parenchyma cells. All the data suggest that the transcript levels of some, but not all genes of the Nramp or mt family are elevated under heavy metal stress. AMF colonization results in a down-regulation of these genes, presumably due to the fact that the content of heavy metals is lower in mycorrhizal than in non-colonized roots. A suppression subtractive hybridization (SSH) library from hyphae of the AMF G. intraradices grown in high versus low Zn++ provided none of the genes which were down-regulated at the plant side (mt or Nramp genes). In contrast, several gene sequences coding for enzymes potentially catalysing the detoxification of reactive oxygen species were found. Thus the fungal cells in the symbiosis may primarily have to cope with the heavy metal-induced oxidative stress.

Journal of Psychiatric Research (1)


http://www.sciencedirect.com/science/article/B6T8T-49H1HKV-4/2/3d6cb50b9a82fb94fd4a08e5228ee5bd

Extensive animal studies suggest neuropeptide Y (NPY) to be involved in coping with a wide range of stressors, and that impaired central NPY signalling could be involved in the pathophysiology of anxiety and depression. Human studies of central NPY levels in depression have, however, been inconclusive. Here, we examined levels of NPY-like immunoreactivity (NPY-LI) in the cerebrospinal fluid (CSF) of medication-free subjects with treatment refractory unipolar depression. Patients were admitted to a research inpatient unit, examined under standardized conditions, and compared with a sample of volunteers in whom psychiatric morbidity was excluded. A robust suppression of NPY levels in patient CSF was found, while other putative CSF markers (monoamine metabolites, somatostatin) did not differ between the groups. We then explored whether this finding might be related to a recently described T1128C coding polymorphism which results in a Leu7-> Pro7 substitution of the signal peptide, and a previously not described T -399C polymorphism in the promoter region of the preproNPY gene. Preliminary evidence was found for an association of both markers with a diagnosis of depression, indicating...
the possibility of an underlying haplotype influencing the vulnerability for developing depressive illness. Our present findings are in line with an extensive animal literature, and further support the notion that impaired NPY function could contribute to depressive illness.

**Journal of Reproductive Immunology** (4)


http://www.sciencedirect.com/science/article/B6T8W-48M7VYN-5/2/bb92e190d0abb5e883c4626b995548f3

The placenta acts as an immunological barrier between the mother and the fetal "graft", allowing two antigenically different organisms to tolerate one another. In placentae from preeclamptic women, we have demonstrated, by an ultrastructural assessment and an immunohistochemical study, a placental barrier breakage leading to the mixing of maternal and fetal antigenically different blood. This condition could be responsible for the triggering of a maternal rejection reaction that we presume to be at the basis of the preeclamptic syndrome. Thus, we have investigated the Human Leukocyte class II DR antigens (HLA-DR), whose role in self and non-self recognition is well known, in women with preeclampsia, their partners and in control couples using the serological Terasaki technique. The results showed a statistically significant increase of HLA-DR homozygosity and a reduced antigenic variety in the preeclamptic women and their partners with respect to controls. In this update, we have examined the 2nd exon of the human gene, HLA-DRB1, on the short arm of the chromosome 6 using DNA sequence-based typing (S-BT) PCR in 56 preeclamptic couples and 64 control couples. The results have confirmed the significant excess of HLA-DR homozygosity in couples associated with preeclampsia versus controls. From our results, it emerges that HLA-DR homozygosity and the reduced antigenic variety seem to be associated to a major risk for preeclampsia, which further appears to be a "couple's disease".


http://www.sciencedirect.com/science/article/B6T8W-3V5TKM2-7/2/3c7a90b4a25564e1b60dbe8ea00d858b

In situ PCR hybridization studies in the testis of infected asymptomatic subjects detected the presence of HIV-1 proviral DNA in the nuclei of germ cells at all stages of differentiation suggesting that HIV-seropositive men produce infected spermatozoa that are released in the genital tract. In all subjects studied spermatogenesis was normal, the presence of provirus was not associated with germ cell damage and a very mild local immune response was observed. The HIV hybridization pattern observed in germ cells supports the hypothesis of a clonal infection. It is suggested the possibility of a direct infection of the germ cells by cell-free virus and that the testis might represent a site of early viral localization, well tolerated because of the immune privilege of this organ.
For a number of years we have intensively investigated the localization of HIV-1 in male genital tract tissues and secretions using a variety of microscopy techniques including immunocytochemistry, in situ hybridization, in situ PCR and electron microscopy. Our studies have failed to demonstrate an association between HIV-1 and either testicular germ cells or spermatozoa. In this article we present our results in the context of other related studies, and discuss the strengths and weaknesses of the techniques that have been used to address this important research question.


Tight control of MHC expression is essential for the outcome of a successful pregnancy. The lack of MHC class II and class I mediated antigen presentation by fetal trophoblast cells is an important mechanism to evade maternal immune recognition. Interestingly, the deficient expression of MHC class II molecules (HLA-DR, -DQ and -DP) and of the classical MHC class I molecules HLA-A and HLA-B is also noted after IFN-[gamma] treatment in trophoblast-derived cell lines. Our studies show that in trophoblast cell lines the IFN-[gamma] induced transactivation of HLA-A and HLA-B promoters is repressed. Furthermore, it was found that trophoblast cells lacked IFN-[gamma] mediated induction of the class II transactivator (CIITA). This lack of CIITA expression in trophoblast cells is due to CIITA promoter hypermethylation. In addition to lack of CIITA expression, trophoblast cells also displayed a repressed expression of RFX5. Together, these observations reveal a silencing of multiple activation pathways that are critical to the transcriptional control of MHC class II and class I antigen presentation functions by trophoblast cells.

Journal of Surgical Research


Background Abdominal aortic aneurysm (AAA) is a common aged disease of human aorta with increasing incidence. It is characterized by dramatic vascular remodeling via proteolysis and degradation of matrix proteins. Integrins are important cellular receptors for matrix proteins, which may have an association with pathological remodeling. The present study was undertaken to
analyze the expression of integrin subunits in human aneurysmal aortas and with healthy aortic tissues as controls. Materials and methods The expression of integrin genes in AAA specimens and healthy human aortic tissues was detected by RT-PCR technique. The correlation of variation and distribution of smooth muscle cells (SMCs) and integrin protein expression in the corresponding tissues were studied immunohistochemically. Results The gene transcripts coding for integrin [alpha]4, [alpha]5, [alpha]V, [beta]1, [beta]3, [beta]5, and [beta]6 subunits were constitutively expressed in the normal aortas. Only gene expressions of integrin [alpha]5 and [beta]1 were significantly decreased by 81% and 85%, respectively, in AAA specimens (P Conclusions The marked decrease in integrin [alpha]5[beta]1 expressions was unique to aneurysmal aortic tissues and correlated to a decrease in density of SMCs, which are the major cells in maintaining the structure stability of normal aortas. As integrin [alpha]5[beta]1 specifically binds fibronectin and collagen, those results may suggest that the absence of integrin [alpha]5[beta]1 activity impaires matrix protein attachment and alter the architecture in aortic media thereby lead to the deformity of aorta and aneurysm formation.


http://www.sciencedirect.com/science/article/B6WM6-4CTCV63-F/2/7cd716cf028a4cbec7e0b82fc7203301

Background Hereditary diffuse gastric cancer (HDGC) is a disease mediated by down-regulation of the tumor suppressor E-cadherin (CDH1). This disease is particularly dangerous because of the youth of the patients, and for clinical management, hampered by the submucosal spread of tumor invisible at endoscopy. Two mechanisms of CDH1 down-regulation have been described in HDGC: missense mutations in the CDH1 gene and gene silencing through promoter methylation. Materials and methods Seven patients affected by HDGC were enrolled. Tumor tissues were checked for CDH1 expression by immunohistochemistry (IHC). CDH1 DNA sequencing was performed for all its 16 exons from tumor and normal tissues of the same patients to detect somatic and germ-line mutations. Methylation promoter study was performed using specific primers and PCR. Results IHC analysis confirmed CDH1 down-regulation in all patients. DNA sequencing revealed the presence of six missense mutations in five patients. Four mutations were at the EC-3 domain of CDH1, whereas the other two were found in the cytoplasmic region interacting with catenins. All six mutations were absent in normal tissue, thereby excluding its presence in germ-line cells. Four patients exhibited both DNA missense mutations and gene silencing through promoter methylation. In two patients we did not notice either DNA missense mutations or promoter methylation. Conclusion CDH1 somatic mutations and promoter methylation synergistically induce CDH1 down-regulation in HDGC patients, whereas germ-line mutations are relatively rare. However, other unknown mechanisms of CDH1 suppression are involved to explain CDH1 down-regulation in HDGC patients without CDH1 mutations and promoter methylation.


http://www.sciencedirect.com/science/article/B6WM6-49D6P0F-4/2/36f963684e96fb1e970ead996330283f

Background The aim of this study was to understand the role of ischemic preservation injury and pro-inflammatory cytokine expression in the progression of allograft vasculopathy. Methods Using the rat aortic transplant model, grafts were stored at 4[deg]C for either 1 or 24 h. Graft vasculopathy was assessed at 4 and 8 weeks after transplantation. Intra-graft cytokine
expression was measured at days 1, 3 and, 7 after transplantation. Results At 4 weeks, intimal hyperplasia of allografts was greater than isografts (P P P P). Conclusions Prolonged ischemic preservation injury induced vascular disease in both isografts and allografts. The vessel wall reaction increased over time and was greater in allografts than isografts. The enhanced expression of T cell- and macrophage associated cytokines in allografts compared to isografts, suggested that early pro-inflammatory cytokine expression played an important role in progression of allograft vasculopathy.


http://www.sciencedirect.com/science/article/B6WM6-4DS45W9-6/2/8fb09387391d5143bb21550c9a6a1f20

Background Manganese superoxide dismutase (MnSOD) plays a critical role in the detoxification of mitochondrial reactive oxygen species, constituting a major cellular defense mechanism against agents that induce oxidative stress. A genetic polymorphism in the mitochondrial targeting sequence of this gene has been associated with increased cancer risk. This one base pair transition (-9 T>C) leads to a Val to Ala amino acid change in the mitochondrial targeting sequence. In addition, the MnSOD promoter contains an activator protein-2 (AP-2) binding site that modifies transcription of MnSOD. Mutations have been identified in the proximal region of the promoter in human tumor cell lines. One of these mutations (-102 C>T) has been shown to change the binding pattern of AP-2, leading to a reduction in transcriptional activity. The aim of our study was to investigate possible associations of the (-9 T>C) and (-102 C>T) polymorphisms with gastric cancer in a population-based case-control study conducted in Warsaw, Poland. Materials and methods DNA was obtained from a population based case-control study of stomach cancer conducted in Warsaw, Poland, between 1994 and 1996. The MnSOD -9 T>C genotype was determined by PCR-RFLP assay. The MnSOD -102 C>T genotype was determined using a TaqMan allele discrimination assay. Results The frequency of the -102 C>T polymorphism was 41% (38/91) in gastric cancer cases and 38% (50/130) in the controls (odds ratio [OR] 1.1, 95% confidence interval [CI] 0.6-2.1). The frequency of the -9 T>C polymorphism was 44% (202/464) in cases and 56% (262/464) in controls (OR 1.1; 95% CI 0.9-1.37). The lack of association was observed in both non-smokers (OR 1.5; 95% CI 0.7-2.34) and smokers (OR 1.1; 95% CI 0.7-1.7). Furthermore, the association was not significant when smokers were segregated by extent of smoking history. Conclusion The association of the manganese superoxide dismutase polymorphisms at -102 C>T and the -9 T>C were not found to be associated with gastric cancer in a Polish case-control study.

Journal of the American College of Cardiology (7)


http://www.sciencedirect.com/science/article/B6T18-4CRXMCG-14/2/22fa828d6747f2122439d18feb24e20b
Objectives This study was designed to describe the frequencies of multiple myocardial infarction (MI) risk-associated genotypes among different racial groups.

Background Racial disparities in the prevalence of cardiovascular disease (CVD) are well known. Recent large Japanese case-control studies identified connexin-37 (GJA-4), plasminogen activator inhibitor-1 (PAI-1), and stromelysin-1 (MMP-3) polymorphisms as risk factors for MI, but the prevalence of these genotypes among different racial groups in the U.S. needs to be determined.

Methods Genomic deoxyribonucleic acid from 95 healthy African Americans (AA) and 95 healthy European Americans (EA) was used for genotyping. Deoxyribonucleic acid containing the region of interest was amplified using the polymerase chain reaction, followed by genotyping using pyrosequencing.

Results All three MI-risk genotypes were observed in both populations and were in Hardy-Weinberg equilibrium. The frequencies of two of the three "risk-associated" genotypes were significantly higher in the AA population: GJA4 C1019T T/T: AA, 20%, EA, 7% (p = 0.053); MMP3 -1171delA A/A: AA, 78%, EA, 24% (p = 0.018).

Conclusions We found higher frequencies of disease-associated genotypes in AA than in EA. Our results also show that more AA than EA carry multiple risk-associated genotypes. Future studies need to assess whether such genetic profiles predict adverse outcomes in U.S. populations and contribute to racial disparities in CVD burden.


http://www.sciencedirect.com/science/article/B6T18-4DFJ DW5-Y/2/592dddf89f0c0bdc2b6fd3745eb18b1

Objectives We sought to determine the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) induction on post-myocardial infarction (MI) remodeling, especially in relation to the inflammatory response and myocardial fibrosis.

Background Granulocyte-macrophage colony-stimulating factor modifies wound healing by promoting monocytopoiesis and infiltration of monocytes and macrophages into injured tissue; however, the effect of GM-CSF induction on the infarct healing process and myocardial fibrosis is unclear.

Methods A model of MI was produced in Wistar rats by ligation of the left coronary artery. The MI animals were randomized to receive GM-CSF inducer (romurtide 200 μg/kg/day for 7 consecutive days) (MI/Ro) or saline (MI/C).

Results Echocardiographic and hemodynamic studies on day 14 revealed increased left ventricular (LV) end-diastolic dimension, decreased fractional shortening, elevated LV end-diastolic pressure, and decreased LV maximum rate of isovolumic pressure development in MI/Ro compared with MI/C. Immunoblotting showed that expression of transforming growth factor (TGF)-[beta]1 in the infarcted site on day 3 after MI was decreased in MI/Ro compared with MI/C. In the infarcted site, TGF-[beta]1, collagen type I and type III messenger ribonucleic acid (mRNA) expression on day 3, and collagen content on day 7 were reduced in MI/Ro compared with MI/C, in association with marked infarct expansion. In MI/Ro, monocyte chemoattractant protein-1 mRNA level and the degree of infiltration of monocyte-derived macrophages (ED-1-positive) were greater in the infarcted site on day 7 than those in MI/C.

Conclusions The GM-CSF induction by romurtide facilitated infarct expansion in association with the promotion of monocyte recruitment and inappropriate collagen synthesis in the infarcted region during the early phase of MI.


http://www.sciencedirect.com/science/article/B6T18-48VWB6W-13/2/9e63bb568aa2fd42644186c27e8f057b
Objectives
Given the importance of endothelial nitric oxide synthase (eNOS) in regulating endothelium-dependent vasorelaxation, we investigated the effects of high-density lipoprotein in (HDL) on eNOS protein abundance in cultured human vascular endothelial cells. Background: Endothelial dysfunction, characterized by decreased nitric oxide production, is one of the early features in the development of atherosclerosis. We have recently shown in vivo that niacin therapy increases plasma HDL concentration and improves endothelium-dependent vasorelaxation in patients with coronary artery disease. Methods: Human vascular endothelial cells were cultured in the presence or absence of HDL or apolipoprotein (apo)A-I. The eNOS protein abundance was assessed by immunoblotting, and protein half-life was assessed by pulse-chase techniques. The eNOS messenger ribonucleic acid (mRNA) abundance was measured using real-time quantitative polymerase chain reaction. Results: High density lipoprotein, or apoA-I alone, increased eNOS protein abundance by 3.5 +/- 0.7 and 2.7 +/- 0.5-fold, respectively (p < 0.05). Conclusions: We demonstrate that HDL activates both extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, resulting in enhanced eNOS protein stability and subsequent accumulation of eNOS protein. This posttranslational regulation represents a previously unrecognized mechanism for regulating eNOS.


Objectives
We examined whether selective cyclooxygenase-2 (COX-2) inhibition in apolipoprotein-E (apoE) deficient mice reduces cytomegalovirus (CMV) replication, and determined whether COX-2 anti-inflammatory activity leads to decreased atherosclerosis. Background: Evidence suggests that CMV infection contributes to atherosclerosis and that this occurs in part through inflammatory mechanisms. Cyclooxygenase-2 inhibitors are potent anti-inflammatory agents. They also inhibit CMV replication in vitro. Methods: The apoE deficient mice were either treated or not treated with a selective COX-2 inhibitor, and either infected or not infected with CMV. Viral deoxyribonucleic acid load in salivary glands was determined by quantitative polymerase chain reaction. Atherosclerotic lesion analysis was performed by standard methods. Results: In vivo COX-2 inhibition, unexpectedly increased viral load: in the CMV-infected animals viral load was 2.58 +/- 1.0 in the nontreated group, 4.74 +/- 1.38 in the group treated with 12 mg/kg/day MF-tricyclic, and 6.51 +/- 1.64 in the group treated with 24 mg/kg/day MF-tricyclic (p TREND = 0.050). This increased viral load was paralleled by increased anti-CMV antibody titers. Most surprisingly, COX-2 inhibition significantly increased early atherosclerotic lesion area, independent of viral infection. Conclusions: Our study demonstrates that selective inhibition of COX-2 in vivo increases viral load. The finding that inhibition of COX-2 increases atherosclerosis development in apoE deficient mice suggests, unexpectedly, that this enzyme exerts antiatherosclerosis activity, at least in this model.


http://www.sciencedirect.com/science/article/B6T18-45JG5R2-V/2/a50824b196aded16025e642bdf8f4628

Objectives
This study was designed to investigate the roles of Fas/FasL pathway in myocardial damage in murine acute myocarditis caused by Coxsackie virus B3 (CVB3). Background: Cardiac...
myocyte apoptosis rarely occurs in murine acute myocarditis caused by CVB3. Fas/FasL belong to the tumor necrosis factor receptor/ligand superfamily of costimulatory molecules and are known to play a critical role in the induction of apoptosis, as well as in the cytotoxicity mediated by T-cells and natural killer cells.

**Methods**

We first analyzed the expression of Fas on cardiac myocytes in vivo and in vitro. Second, we examined the development of myocardial damage, in C3H/He mice treated with an anti-FasL monoclonal antibody (mAb), and in C3H/He-lpr/lpr mice and C3H/He-gld/gld mice infected with CVB3. Third, to investigate the effects of anti-FasL mAb treatment on the activation of the infiltrating cells, we examined the expression of interferon (IFN)-gamma and interleukin (IL)-2 as activation markers in the heart of mice by semiquantitative polymerase chain reaction.

**Results**

Fas was markedly induced on cardiac myocytes with acute myocarditis. Myocardial inflammation was decreased in mice treated with anti-Fas L mAb, C3H/He-lpr/lpr mice and C3H/He-gld/gld mice. Anti-FasL mAb-treatment also decreased the expression of IFN-gamma, IL-2, inducible nitric oxide synthase and CVB3 genomes in myocardial tissue.

**Conclusions**

Our findings strongly suggested that the Fas/FasL pathway played a critical role in the development of massive myocardial necrosis through activation of infiltrating cells, and raise the possibility of immunotherapy by blocking the Fas/FasL pathway to prevent myocardial damage and improve the prognosis of patients with viral myocarditis.

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**References**


http://www.sciencedirect.com/science/article/B6T18-42VM8NN-4/2/d1a318e6e10b6f975142a43157efb60f

OBJECTIVES

Our aim was to study whether an insertion/deletion (I/D) polymorphism in the [alpha]2B-adrenoceptor gene is associated with the risk for cardiovascular diseases. BACKGROUND [alpha]2-adrenoceptors mediate contraction of vascular smooth muscle and induce coronary vasoconstriction in humans. The [alpha]2-adrenoceptor subtype B mediates vasoconstriction in mice. A variant of the human [alpha]2B-adrenoceptor gene that encodes a D of three residues in an intracellular acidic motif has been shown to confer decreased receptor desensitization. This receptor variant could, therefore, be involved in diseases associated with enhanced vasoconstriction. METHODSThis study was part of a prospective population-based study investigating risk factors for cardiovascular diseases in a cohort of middle-aged men from eastern Finland. Nine hundred twelve men aged 46 to 64 years were followed for an average time of 4.5 years. RESULTSIn this study population, 192 men (21%) had the D/D genotype; 256 (28%) had the I/I genotype, and 464 (51%) had a heterozygous genotype. In a Cox model adjusting for other coronary risk factors, men with the D/D genotype had 2.2 times (95% confidence interval: 1.1 to 4.4, P = 0.02) the risk to experience an acute coronary event (n = 15 for D/D, 10 for I/I and 12 for I/D) compared with men carrying either of the other two genotypes. The [alpha]2B-adrenoceptor genotype was not associated with hypertension in this study population. CONCLUSIONSThe D/D genotype of the [alpha]2B-adrenoceptor is a novel genetic risk factor for acute coronary events, but not for hypertension.


http://www.sciencedirect.com/science/article/B6T18-4C8G0WD-D/2/0a1a5579de6369ece47c3cb341d952e4

**Objectives**

We evaluated whether the angiotensin II (Ang II) receptors from perioperation through
Background

The role of Ang II receptors (type 1: AT1R; type 2: AT2R) in TCAD is uncertain.

Methods

We investigated 28 heart donors and the corresponding recipients. The levels of AT1R and AT2R messenger ribonucleic acid (mRNA) were examined in lymphocytes from the donor spleen and in the donor heart at one-week and one-year posttransplantation to determine their association with the progression of TCAD, measured as changes in maximal intimal thickness (CMIT) and plaque volume (CPV) by intravascular ultrasound (IVUS) examinations.

Results

The AT1R mRNA in lymphocytes from the donor spleen (CMIT: R = 0.73, p < 0.05) and AT2R mRNA in the donor hearts at one-year post-transplantation (CMIT: R = 0.3, p = 0.05) proved to be multivariate predictors of the progression of TCAD.

Conclusions

These data suggest a role for Ang II receptors in the pathogenesis of TCAD and support a novel concept that TCAD may have its origin in the donor per se and may be modulated by the recipient's inherent biological factors.

Journal of the American College of Surgeons (2)


http://www.sciencedirect.com/science/article/B6T91-42G0MFJ-5/2/cb9d5e7f504a3a35eeef7488b82d77b9c

Background: The mechanisms of the reported high increase in interleukin-6 (IL-6) levels after esophagectomy are unclear. We investigated the influence of an intrathoracic procedure, esophagectomy, on IL-6 production in lung tissue.

Study Design: Fourteen paired lung tissue samples were obtained from patients before and after they underwent transthoracic esophagectomy for esophageal cancer. IL-6 levels in the lung were measured with enzyme-linked immunosorbent assay, and IL-6 mRNA expression was determined with real-time quantitative reverse transcription-polymerase chain reaction. Immunohistochemical staining was used to localize IL-6, and circulating levels were also measured.

Results: IL-6 protein and mRNA were significantly increased in lung tissue after this intrathoracic procedure (p < 0.05).

Conclusions: Transthoracic esophagectomy causes an increase in IL-6 production from airway epithelial cells, secondary to increased expression of IL-6 mRNA. Local response of lung tissue may be one source of increased serum IL-6 after this procedure.


http://www.sciencedirect.com/science/article/B6T91-4FJT8T9-6/2/cba5a2216fcfc27129b32e6d18a0fc1b

Background: To find out if neoadjuvant therapy could alter tumor response determinants that might affect tumor sensitivity to the treatment, we investigated intratumoral expressions of genes associated with chemosensitivity, radiosensitivity, or both before and after radiochemotherapy.

Study design: Twenty-four patients with locally advanced, resectable esophageal cancer (cT2-4, Nx,M0) received neoadjuvant 5-FU/cisplatin/36 Gy treatment followed...
by transthoracic en bloc esophagectomy. Expression levels of thymidylate synthase, dihydropyrimidine dehydrogenase, excision repair cross-complementing gene 1, glutathione S-transferase Pi, epidermal growth factor receptor, and HER2 were measured in matched preradiochemotherapy endoscopic tumor biopsies and in postradiochemotherapy resection specimens. mRNA was isolated from formalin-fixed, paraffin-embedded, laser microdissected tumor tissues, and a quantitative fluorescent dye real-time reverse transcription polymerase chain reaction system was used for gene expression measurement. Results There was a significant reduction in the expression levels of thymidylate synthase (p

Conclusions The expression levels of a set of genes that are possible determinants of 5-FU/cisplatin/radiation therapy antitumor activity are significantly downregulated by neoadjuvant radiochemotherapy in esophageal cancer.

Journal of the American Society for Mass Spectrometry (2)


http://www.sciencedirect.com/science/article/B6TH2-48CX6M7-4/2/afe37edd9f93709f981110b7b967ffcc

The advantages of the thermostable DNA polymerase from Thermococcus kodakaraensis (KOD) are demonstrated for PCR amplification with subsequent detection by mass spectrometry. Commonly used DNA polymerases for PCR amplification include those from Thermus aquaticus (Taq) and Pyrococcus furiosus (Pfu). A 116 base-pair PCR product derived from a vWA locus was amplified by Taq, Pfu, or KOD DNA polymerase and compared by agarose gel electrophoresis and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). KOD DNA polymerase demonstrated a 2- to 3-fold increase in PCR product formation compared to Pfu or Taq, respectively, and generated blunt-ended PCR product which allows facile interpretation of the mass spectrum. Additionally, we demonstrate the advantage of using high magnetic fields to obtain unit resolution of the same 116 base pair (~72 kDa) PCR product at high m/z.


Several investigators have observed a discrepancy in electrospray response of complementary strands from denatured DNA, which has been attributed to the difference in hydrophobicity between the two strands; the more hydrophobic species tend to have higher ion abundances. The implementation of a heated electrospray source has allowed us to "level" the electrospray response for two equimolar complementary strands with different hydrophobicities. As the temperature was increased, the ratio of ion abundances of the less hydrophobic noncoding strand to the more hydrophobic coding strand approached unity. Furthermore, the heated electrospray source was used to denature amplicons containing 7-deaza purines, which can be used to facilitate sequencing by mass spectrometry.

http://www.sciencedirect.com/science/article/B75DF-4CWYXY8-7/2/68b585b938a4cd8655bedef2a9530e90

We describe three new automated methods for purifying genomic DNA from whole blood. The MagneSil(R) Blood Genomic, Max Yield System uses MagneSil(R) paramagnetic particles (PMPs) in a 96-well format to purify the maximal amount of DNA from a 200-[mu]L blood sample. In contrast, the MagneSil(R) ONE, Fixed Yield Blood Genomic System uses MagneSil(R) Fixed Yield PMPs to purify a normalized amount of DNA from 60 [mu]L of blood in a 96-well format. These methods are implemented on the Beckman Coulter Biomek(R) FX automated workstation. The MagneSil(R) KF Genomic System uses MagneSil(R) PMPs to purify DNA from 1 to 15 samples of 200-[mu]L blood using the moderate-throughput Thermo Electron KingFisher(R) mL instrument. The MagneSil(R) Blood Genomic System typically yields > 4 [mu]g per 200 [mu]L of whole blood, depending on the white blood cell content. The MagneSil(R) ONE System is best suited where there is a requirement for purification of a narrow concentration range of DNA. This system purifies 1 [mu]g (+/-50%) of DNA from 60 [mu]L of blood. The MagneSil(R) KF System purifies 2 to 6 [mu]g of DNA from 200 [mu]L of blood. DNA purified using all of these methods is suitable for PCR, STR, READIT(R) SNP genotype analysis, and multiplexed PCR analysis.


http://www.sciencedirect.com/science/article/B75DF-4DGSX88-F/2/f94319647f53ba0f2942cc81a6f7de13

A critical need exists for the development of next-generation genomic analysis instrumentation capable of offering significantly higher throughput at a lower cost than current technology. In this paper, we explore the potential of natural convection-based systems to address these issues by providing a thermocycling hardware platform capable of performing rapid polymerase chain reaction (PCR) amplification of DNA. These systems can be arrayed in a multi-well format that is simple to operate, is suitable for integration with high-throughput automated liquid handling systems, and can be easily and inexpensively mass-produced.

A cDNA clone has been isolated from a human hippocampal cDNA expression library by relying on the selectivity of two antisera that are specific for imidazoline binding proteins. A 1789 bp cDNA clone was sequenced and shown to contain a single open-reading frame that predicts a 66 kDa polypeptide, but it is truncated based on its lack of a stop codon and poly-A+ tail. Two regions of homology exist for the predicted amino acid sequence in common with chromogranin-A and B proteins, a zinc finger protein, and the ryanodine receptor. Northern blot analyses of poly-A+ mRNA from 36 human tissues indicated two differentially expressed transcripts of 6.0 and 9.5 kb. The 6.0 kb mRNA form was enriched in brain and endocrine tissues as compared to other tissues, but not in strict concordance with I1-imidazoline binding sites. The highest overall amounts of the combined transcripts were found in pituitary. In situ hybridization histochemistry revealed an enrichment of the message in neuronal cell bodies of the rat hippocampus and cerebellar cortex. This clone has some of the properties expected of an imidazoline receptor.


http://www.sciencedirect.com/science/article/B6T92-3YS87RM-12/2/32edbaf43861c3f336f15407ba83573f

The presence and distribution of neuromessenger molecules and receptor mRNA in human trigeminal ganglion was studied with immunocytochemical, in situ hybridisation and RT-PCR techniques. Immunofluorescence staining revealed that calcitonin gene-related peptide (CGRP) immunoreactive (ir) neurons occurred in high numbers, constituting 36-40% of all nerve cell bodies in the ganglion. Accordingly, in situ hybridisation demonstrated CGRP mRNA in a large portion of the trigeminal neurons. A small number of the nerve cell bodies showed substance P (SP)-ir, (18%), nitric oxide synthase (NOS)-ir (15%), and pituitary adenylate cyclase activating peptide (PACAP)-ir (20%). Double immunostaining revealed that only few CGRP-ir neurons also were NOS-ir (less than 5%). The C-terminal flanking peptide of neuropeptide Y, C-PON, was not visible in any of the nerve cell bodies studied. Agarose gel electrophoresis of the RT-PCR products from the ganglia demonstrated the presence of mRNA corresponding to CGRP1, NPY Y1 and Y2, and VIP1 receptors. These results suggest both sympathetic and parasympathetic influence on the activity in the trigeminal ganglion.
Objective Detection of HPV DNA in oral and genital lesions of a heterosexual male, 4 months after oral and vaginal intercourse with a woman with vulvar warts. Possible modes of acquisition of oral HPV infection in the male sexual partner are discussed. Setting Genitourinary Medicine clinic. Methods Polymerase chain reaction amplification of genomic DNA from oral and genital lesions. HPV DNA typing by dot blot hybridization. Results HPV DNA types 6 and 11 were identified in a polypoid tongue lesion and in a penile wart from the male sexual partner. Conclusions The acquisition of oral HPV infection in the male sexual partner may have resulted from genital-oral HPV transfer, either by direct contact with vulvar warts or by digital self-inoculation.

Journal of the Neurological Sciences (25)


http://www.sciencedirect.com/science/article/B6T06-43P3TH0-5/2/00060d51d9f36eff1cd4e1c970bff0eb

Around 70% of Charcot-Marie-Tooth 1 (CMT1) cases are caused by a dominantly inherited 1.5-Mb duplication at 17p11.2-12 (CMT1A). Using photostimulated luminescence (PSL) imaging of MspI Southern blots, hybridization signals of the probe pVAW409R3a in relation to cohybridized probe SF85a, were densitometrically quantified and an RFLP allele-band ratio determined. A total of 55 Norwegian CMT patients and 16 asymptomatic family members from 26 separate families, clinically and neurophysiologically classified as CMT1 (n=46) and CMT2 (n=9), were studied. Thirty-two of 46 CMT1 cases (69.6%), all heterozygous but one homozygous for the pVAW409R3a MspI polymorphism, from 12 of 21 families (57.1%) were positive for the CMT1A duplication. In autosomal dominant familial cases (n=30), 26 of 30 cases (86.7%), all heterozygous, from six of seven families (85.7%) were positive for duplication. None of the CMT2 patients, asymptomatic family members or healthy controls were positive for duplication. The CMT1A frequency of duplication in Norwegian CMT1 patients is in general agreement with those reported in other European countries and the present results show that quantitative densitometric PSL imaging is a highly reliable test in diagnosing CMT1A duplication.


http://www.sciencedirect.com/science/article/B6T06-44BM9KY-C/2/e19f34509d3a0206c5a9d73ce0f28a3c

We studied a 57-year-old female patient with clinical and biochemical evidences of McArdle's disease. Her muscle biopsy also revealed signs of mitochondrial proliferation, scattered RRF, and a deficit in complex I of the respiratory chain. Molecular genetic analysis showed that the patient was heterozygous for the most common mutation at codon 49 in the myophosphorylase gene. Mitochondrial DNA analysis of muscle tissue revealed an additional G-to-A transition at nucleotide position 7444 in the cytochrome c oxidase subunit I (COI) gene.

http://www.sciencedirect.com/science/article/B6T06-3RSFG7M-H/2/b5b8e086e8c597b99f814412e4dddf1d

It has recently been claimed (Ferrante et al., 1995. HTLV tax-rex DNA and antibodies in idiopathic amyotrophic lateral sclerosis. J. Neurol. Sci. 129 (Suppl.) 140-144) that human T-lymphotropic virus (HTLV) tax-rex sequences are detectable in the peripheral blood mononuclear cells (PBMCs) of 40% of patients with motor neurone disease (MND). In an attempt to confirm this we employed a highly sensitive 'nested' polymerase chain reaction (PCR) assay, capable of detecting single molecules of HTLV proviral DNA, to look for tax-rex sequences in the PBMCs of 43 patients with MND. We were unable to detect the presence of HTLV tax-rex in any of 43 MND patients tested, using three different PCR primer sets under both high and low stringency conditions. Using the same DNA samples we were able to detect the presence of the single-copy pyruvate dehydrogenase gene, thus demonstrating that the extracted DNA was indeed amplifiable by PCR. To further exclude the possibility that the extracted DNA samples contained unrecognised inhibitory factors we conducted spiking experiments with trace amounts (approximately 10 copies) of HTLV proviral DNA. Spiked samples yielded PCR products of the expected size. We are therefore unable to confirm the presence of HTLV tax-rex sequences in this disease.


http://www.sciencedirect.com/science/article/B6T06-485RNBW-JT/2/3ed21d2856150c8fcede6e9fb8c3541b53

The analysis of human skeletal muscle mitochondria revealed a progressive decline in mitochondrial respiratory chain function with age. The activities affected to the greatest extent were those of complexes I and IV which were decreased by 59% and 47% respectively between the ages of 20-30 years and 60-90 years of age. Quantitation of the 5 kb 'common' deletion of mtDNA using PCR revealed a progressive accumulation with age, from approximately 1 in 100 000 at 21 years to 1 in 10 000 at 56 years and 1 in 5000 at 78 years of age. The low absolute levels of this mutation are unlikely to contribute significantly to the observed mitochondrial dysfunction.


http://www.sciencedirect.com/science/article/B6T06-3R8617F-2M/2/42d17e0ee4af598e8ebfae532b04d31

The apolipoprotein E gene (APOE), located on human chromosome 19, has three common alleles ([e]2, [e]3, [e]4) which encode for the three main isoforms indicated as E2, E3 and E4 respectively. Several findings indicate [e]4 allele as an important risk factor in both sporadic and familial late-onset Alzheimer's disease (AD). Pathological changes similar to AD are seen in
almost all patients with Down's syndrome (DS) aged over 35 (senile plaques, neurofibrillary
tangles and neuronal loss); a proportion of these may subsequently develop dementia. Aim of this
study is to evaluate the possible pathological role of [epsi]4 allele as risk factor for developing AD
in a DS population. Apoe [epsi]4 allele frequency is not significantly different in DS cases and
controls. We found a statistically significant inverse correlation between full scale IQ values and
age of patients in the subgroup of DS subjects selected for the presence of at least one [epsi]4
allele, while no correlation was observed in DS subjects with other ApoE genotypes. A
longitudinal analysis of cognitive performances (available in 38 patients) showed a faster rate of
decline in intellectual ability in those subjects carrying at least one [epsi]4 allele. Our data support
the hypothesis that ApoE [epsi]4 allele has a contributory role in accelerating the mental
deterioration of AD-type in DS patients. (c) 1997 Elsevier Science B.V.

patients are 5% of childhood-onset dystrophin-normal muscular dystrophy and most partial
deficiency patients do not have gene mutations." Journal of the Neurological Sciences 140(1-2):
30.

[alpha]-Sarcoglycan (adhalin), a 50-kDa component of the dystrophin-associated complex of
proteins, participates in the stabilization of the myofiber plasma membrane in the membrane
cytoskeleton. Deficiencies of [alpha]-sarcoglycan cause a subset of childhood-onset muscular
dystrophy (SCARMD) cases. However, secondary deficiencies of [alpha]-sarcoglycan are
common. To begin to establish the rates of false positives (secondary deficiencies), we used
immunofluorescence to screen 30 Italian dystrophin-normal muscular dystrophy patient biopsies
and identified 4 patients with partial [alpha]-sarcoglycan deficiency and 2 patients with complete
deficiency. The entire [alpha]-sarcoglycan gene was screened for mutations using RT-PCR and
SSCP of messenger RNA isolated from muscle biopsies in each of the six patients. Aberrant
SSCP conformers and novel mutations were found only in the two complete
immunohistochemical deficient patients. One patient was homozygous for a R34H amino acid
substitution, while the other was a compound heterozygote (R77C, D97G). These three missense
mutations, with additional mutations we and others have previously described, are all localized in
the extracellular domain of [alpha]-sarcoglycan, and most result in the loss or gain of a positively
charged amino acid. These data have strong implications for structure/function maps of the
[alpha]-sarcoglycan molecule. Our results suggest that most patients showing partial [alpha]-
sarcoglycan deficiency exhibit this as a secondary consequence of genetically distinct disorders.
In support of this, we show biochemical data indicating that secondary deficiency patients show
decreased immunostaining with antibodies directed against [alpha]-sarcoglycan, while having
nearly normal quantities of [alpha]-sarcoglycan protein on immunoblot. This data also suggests
that approximately 5% of childhood-onset dystrophin-normal muscular dystrophy patients will
show a primary [alpha]-sarcoglycan deficiency.

of quantitative molecular data and histochemical and biochemical profile." Journal of the
Neurological Sciences 123(1-2): 140.

We studied muscle biopsies of 5 patients with Kearns-Sayre syndrome and 3 patients with
chronic progressive external ophthalmoplegia all with the common deletion. Steady state levels of
normal and deleted mitochondrial DNA (mtDNA) measured in each patient by quantitative PCR were correlated with histochemical and biochemical features. We found that (1) normal mtDNA levels were higher in many patients than in controls; (2) as levels of deleted mtDNA increased, so did levels of normal mtDNA; (3) cytochrome c oxidase (COX) activity and the percentage of COX negative fibers were both related to the levels of deleted mtDNA; and (4) as percentage of ragged red fibers increased, so did levels of total, deleted and normal mtDNA. The quantity of deleted mtDNA plays a key role in determining the severity of COX deficiency, which is responsible for the overaccumulation of mitochondria in muscle.


http://www.sciencedirect.com/science/article/B6T06-3X10TCY-7/2/2c59515616f4feca2acc70b93dd916be

1,25-Dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active form of vitamin D, exerts an immunosuppressive effect and can completely prevent experimental autoimmune encephalomyelitis (EAE). 1,25(OH)2D3 exerts most of its actions only after it has bound to its specific nuclear receptors. To investigate the possible role of vitamin D receptor gene (VDRG) polymorphism in susceptibility to or disease-modulation of MS, we evaluated 77 Japanese patients with 'conventional' MS and 95 controls. A VDRG allelic polymorphism was assessed by Bsm1 endonuclease restriction after specific PCR amplification. Genotypic polymorphism was clearly defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygous). We found overexpression of the b allele (92.9 vs. 84.2%; P=0.0138) and homozygote bb (85.7 vs. 71.6%; P=0.0263) in MS patients compared with controls. The results indicate for the first time an association of MS with VDRG polymorphism, which may be involved in pathogenesis of MS, or in the linkage disequilibrium of VDRG to another pathogenic gene loci. The role of VDR gene polymorphism should be further studied in other populations, and the distribution of other polymorphism, such as Apa I, Taq I, should be also analyzed to confirm another susceptibility gene for MS and to obtain more adequate strategies for treatment of MS.


http://www.sciencedirect.com/science/article/B6T06-43CR9GC-2/2/1b7add39f08243a6a9732a5852225d8f

In Lewis rats, treatment with high doses of cyclosporin A (CsA) suppresses clinical signs of experimental autoimmune encephalomyelitis (EAE), although disease occurs when treatment is ceased. Treatment with low doses of CsA causes EAE to take a chronic relapsing course. We have previously shown that CsA treatment causes a decline in the number of T cells and increased inflammatory cell apoptosis in the spinal cord. The present study was undertaken to assess whether CsA therapy also modulates cytokine mRNA expression by inflammatory cells in the spinal cord of rats with EAE, looking for changes that might contribute to the observed effects of CsA on the course of EAE. EAE was induced in Lewis rats by inoculation with myelin basic protein and adjuvants. At the peak of neurological signs, on day 14 after inoculation, rats were given a single intraperitoneal injection of saline, or CsA at a dose of 8, 16, 32 or 64 mg/kg. The next day, rats were sacrificed, the spinal cords removed, inflammatory cells were extracted from the cords, and mRNA isolated from these cells. Expression of cytokine mRNA was assessed by
semi-quantitative reverse transcription polymerase chain reaction (PCR) and by quantitative real-time PCR. With both techniques, we found that CsA suppressed the expression of interferon-[gamma] mRNA and interleukin-2 (IL-2) mRNA. With real-time PCR, we found that CsA caused significantly increased expression of transforming growth factor-[beta] mRNA. With the different techniques, we observed no consistent pattern of alteration of expression of interleukin-10 or interleukin-4 mRNA. It is possible that these changes in cytokine mRNA expression contribute to the modulation of the clinical course of EAE that is produced by CsA treatment.


http://www.sciencedirect.com/science/article/B6T06-4BNW2TD-1/2/4438339b85de92037ec1fecd25c6c6f83

The diagnosis of leptomeningeal B-cell malignancies is based on the identification of malignant B cells in the cerebrospinal fluid (CSF). We have established a polymerase chain reaction (PCR) approach to characterize the clonally diverse gene encoding the immunoglobulin heavy-chain (IgH) third complementarity determining region (CDR3) of single B cells. We demonstrate that single-cell PCR is readily applicable to individual cells derived from routine CSF cytospins and is a powerful method to discriminate monoclonal neoplastic from polyclonal reactive B-cell responses. Single-cell PCR analysis, as a new tool for the diagnosis and monitoring of neoplastic meningitis associated with B-cell malignancies, is particularly important if cytology, immunocytochemistry, flow cytometry and automated gene scanning of CSF samples are unable to detect malignant monoclonal proliferation.


http://www.sciencedirect.com/science/article/B6T06-479TPP1-2/2/5cb44b49a1fd7f33976294cf93b95e

We report two families (Family S and Family N) with early-onset parkinsonism in two generations. The mode of inheritance appeared to be autosomal dominant, however, haplotype analysis suggested linkage to chromosome 6q25.2-27, the PARK2 locus, and all affected members were homozygotes in their haplotypes. In Family S, the affected father was married to unaffected mother, who carried one disease-linked haplotype at chromosome 6q25.2-27. In Family N, the unaffected mother carried one disease-linked haplotype. Quantitative PCR amplification analysis revealed exon 3 deletion in Family S and exon 5 deletion in Family N. The age of onset was from 18 to 22 years in Family S and 25 to 42 years in Family N. In both of their hometowns, most people lived in the same districts for many generations and consanguineous marriages had been common. Thus, the carrier state of the parkin gene might have been high in those communities, and marriage of a patient and a carrier is expected to result in autosomal dominant like inheritance. We conclude that PARK2 cannot be excluded even if the mode of inheritance appears as autosomal dominant, when the affected patients are young.

Intracerebral inoculation of the MS strain of herpes simplex virus type 2 (HSV-2) into mice causes an acute encephalitis associated with multifocal demyelination and necrotizing retinitis. We have studied the distribution of latent virus in mice that had recovered from the acute encephalitis. Four weeks or longer after inoculation, HSV-2 could be recovered from the trigeminal ganglia of all mice examined by co-culture of explants in roller tubes. The virus could not be recovered from explants of retina or brain stem. HSV-2 latency associated transcript (LAT) was readily detected in the trigeminal ganglia by reverse transcriptase-PCR more than 4 months after inoculation. LAT was also demonstrated in the brain but this required nested PCR for consistent detection. Both LAT and ICP0 mRNA were detected in brain tissue during the acute encephalitis but, unlike LAT, ICP0 mRNA could not be amplified from the trigeminal ganglia or brain beyond 4 weeks after inoculation of the virus. In situ hybridisation with a double-stranded DNA probe to the ICP0/LAT overlap region of HSV-2 revealed signal in trigeminal ganglion neurons and occasional cells in the brain stem. These findings indicate that HSV-2 introduced by intracerebral inoculation becomes latent in the trigeminal ganglia and that transcription of LAT also persists within the brain.


Diff erential expression of interleukins may influence susceptibility to inflammatory diseases such as MS. IL-1α production is increased in MS patients during acute relapse, IL-2 receptor (IL-2R) secretion correlates with disease activity in several inflammatory disorders and is variable in MS. Both IL-4 and IL-10 expression vary significantly with relapse/remission in MS and IL-9 is postulated to inhibit steroid-induced apoptosis. To examine the influence of interleukin (IL) genes on MS susceptibility and clinical course, gene association studies using separate polymorphic microsatellite markers for il-1α, il-2, il-2rβ, il-4 il-9 and il-10 were performed, incorporating 150-177 relapsing-remitting or secondary progressive MS (RR/SPMS) patients, 100-110 primary progressive (PPMS) patients and 152-210 controls. No significant differences existed in allele frequencies between either MS group and controls for any of the interleukin microsatellite markers studied, nor were statistically significant differences observed in PPMS vs. RR/SPMS for any marker. These data indicate that the IL-1α, IL-2, IL-2R, IL-4, IL-9 and IL-10 genes are unlikely to be susceptibility loci for MS in this population.


A new autosomal dominant syndrome in a Swedish pedigree is described. Five patients were affected with cerebellar ataxia and sensorineural deafness. Four of these patients had symptoms of narcolepsy. Optic atrophy, other neurological abnormalities and psychiatric symptoms developed with increasing disease duration. Three patients had non-neurological disease in addition, including diabetes mellitus in two and hypertrophic cardiomyopathy in one. Autopsy with neuropathological examination was performed in one case. Molecular studies focused on the
short arm of chromosome 6, including the HLA DR2 locus associated with narcolepsy and the 
(CAG)n repeat at the spinocerebellar ataxia type 1 (SCA1) locus. Biochemical investigation of
muscle biopsy of one case indicated mitochondrial dysfunction with selective decrease in ATP
production for substrates that normally give the highest rates. The activity of glutamate
dehydrogenase was reduced, indicating a low mitochondrial density. We postulate an autosomal
dominant genetic factor responsible for this syndrome. Linkage was excluded to HLA DR2, and a
normal sized SCA1 repeat was observed. We conclude that a locus predisposing to ataxia,
deafness and narcolepsy exists outside this region of chromosome 6.

extraocular and limb muscle." Journal of the Neurological Sciences 179(1-2): 76.

http://www.sciencedirect.com/science/article/B6T06-41H3KMT-8/2/fd583026a595a4779d8dc02aa532615a

The extraocular muscles (EOM) are anatomically and physiologically distinct from other striated
muscles in mammals. Among other differences, they can be driven to generate individual twitch
contractions at an extremely high frequency and are resistant to [Ca2+] induced myonecrosis.
While EOM are preferentially targeted in some neuromuscular diseases such as myasthenia
gravis and congenital fibrosis of the extraocular muscles, they are enigmatically spared in
Duchenne’s muscular dystrophy, despite the widespread damage seen in all other skeletal
muscle groups during the course of this disease. To address the molecular mechanisms that
specify the EOM-phenotype, we characterized the transcriptional profile of genes expressed in rat
EOM versus limb muscle using a differential display strategy. Ninety-five putative differentially
expressed cDNA tags were cloned, from which fourteen were confirmed as being differentially
expressed by RNA slot blot and Northern blot analysis. Ten of these cDNAs were homologous to
known human or murine genes and ESTs, while four genes that were upregulated in EOM were
novel, and have been named expressed in ocular muscle (eom) 1-4. The identification of these
differentially expressed genes may provide mechanistic clues toward understanding the unique
patho-physiological phenotype of EOM.

the association with HLA class II alleles." Journal of the Neurological Sciences 177(1): 65.

http://www.sciencedirect.com/science/article/B6T06-4123D0R-8/2/285f1c4bd70f82e21ed26ba3c02c2c08

We have previously reported that the association between Bsm I polymorphism, one of the
vitamin D receptor genes (VDRG) polymorphism, and multiple sclerosis (MS). In this report, we
investigated the further possible role or relevance of VDRG in the pathogenesis of MS. Apa I
polymorphism was detected by PCR-RFLP from the DNA of 77 conventional MS patients and 95
healthy controls. The study of the Bsm I and Apa I haplotypes was carried out by employing
previously reported Bsm I data. The AA genotype and the [A] allele in the profiles were
significantly more prevalent in MS patients than in controls (P=0.0070 and P=0.0321,
respectively). In the [A] allele-positive MS patients, the positive rate of DPB1*0501 in HLA was
significantly higher than that of the [A] allele-positive controls and that of the [A] allele-negative
MS patients even when the corrected P value (Pcorr) was applied (Pcorr=0.0220 and
Pcorr=0.0077, respectively). The frequency of DRB1*1501 was higher in the [A] allele-positive
patients than in the [A] allele-positive controls and the [A] allele-negative patients
(Puncorr=0.0431 and Puncorr=0.0089, respectively), but the P values did not reach statistical
significance after P corrections. The rate of Bsm I and Apa I haplotypes was much higher in
bA/bA-positive MS patients than in the controls (P=0.0003), and in the bA positive MS patients,
the positive rate of DPB1*0501 was higher than that of the bA-positive controls and that of the bA-negative MS patients (Pcorr=0.0308 and Pcorr=0.0033, respectively). These results indicate that VDRG polymorphism may be associated with susceptibility to MS, and HLA alleles may correlate with risk for MS together with VDRG.


http://www.sciencedirect.com/science/article/B6T06-41H3KMT-7/2/648b50c613b61e5a8fe7c092ca82ccce

Estrogen has been reported to have immunosuppressive functions, and to inhibit the progression of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Since estrogen shows its biological effects via estrogen receptors (ER), we investigate the possible role of ER genes (ERG) in the pathogenesis of MS. Pvull and XbaI polymorphisms in ERG were detected by PCR-RFLP from the DNA of 79 conventional MS patients and 73 healthy controls. The [P] allele in the profiles in Pvull was significantly more prevalent in MS patients than in the controls (PXbaI polymorphism, the onset age of MS patients with the Xx genotype was earlier than that of the xx genotype group (mean age+/-S.D.; 22.60+/-.8.04, and 27.49+/-.9.14, respectively) (PPvull polymorphism might be associated with susceptibility to MS, and XbaI polymorphism with onset age of MS. ERG polymorphism should be further studied in other populations to improve strategies for treatment of MS.


http://www.sciencedirect.com/science/article/B6T06-3RSFG7M-J/2/82ea6508ac9ff892a8ead3d3b6706506

Campylobacter jejuni is a major pathogen preceding Guillain-Barre syndrome (GBS), and most C. jejuni isolates from GBS patients belong to Penner serotype 19 (heat-stable; HS-19). We analyzed sixteen independent clinical isolates from GBS patients, twelve of which belonged to HS-19, three to HS-2, and one to HS-4, using PCR-based RFLP analysis of a flagellin-A (flaA) gene. Two isolates from patients with Miller Fisher syndrome (MFS), and 27 from patients with uncomplicated enteritis were also examined. All HS-19 isolates, regardless of GBS, showed an identical pattern (Cj-1) by RFLP typing and were distinguishable from those of the other Penner serogroups. In contrast, HS-2 and HS-4 isolates were divided into several different RFLP groups, suggesting HS-19 strains are genetically distinctive among C. jejuni isolates. A DNA fingerprinting method also failed to detect any specific band pattern for GBS-related C. jejuni isolates. We examined relationships among anti-GM1 antibody titres in the sera of GBS patients, clinical forms of GBS, serotype of C. jejuni, and the presence of GM1-like structures in lipopolysaccharide (LPS) components from C. jejuni isolates by immunoblotting. HS-19 related GBS was significantly associated with elevated anti-GM1 antibody titers in the sera of the patients, but not associated with any clinical pattern of GBS. No significant correlations were found between anti-GM1 antibody and the pattern of disease, or between GBS-related C. jejuni strains and the presence of GM1-like structures. HS-19 strains seem to be unique among C. jejuni isolates, and HS-19-related GBS may provide an excellent model for clarification of the pathogenesis of GBS.

(dSMA-V) and Charcot-Marie-Tooth disease type 2D (CMT2D) segregate within a single large kindred and map to a refined region on chromosome 7p15. *Journal of the Neurological Sciences* **161**(1): 23.

http://www.sciencedirect.com/science/article/B6T06-3V8RP1X-5/2/2bd80fb0c03d8751c1079b0740ffeaa5

Two separate disorders, autosomal dominant distal spinal muscular atrophy type V (dSMA-V) characterized by marked bilateral weakness in the hands and atrophy of thenar eminence and the first interosseous muscle, and Charcot-Marie-Tooth disease type 2D (CMT2D) characterized by sensory deficits in addition to the upper limb weakness and wasting, have been independently linked to chromosome 7p. We identified a multigenerational Mongolian kindred with 17 members affected with either dSMA-V or CMT2D and mapped both syndromes to the same region on chromosome 7p15. A maximum two-point lod score of 4.74 at recombination fraction zero was obtained with marker D7S474. Tight linkage without recombination was also detected with markers D7S526 and D7S632. A multipoint lod score of 6.07 suggested that the gene is located between markers D7S526 and D7S474. A single conserved haplotype was associated with dSMA-V and CMT2D. Based on informative recombination events, the disease locus was placed between markers D7S516 and D7S1514 within the 7p15 band. Data obtained from this study suggest that a single gene is responsible for both syndromes, dSMA-V and CMT2D, and extend our knowledge of the candidate region.


http://www.sciencedirect.com/science/article/B6T06-485RJ8W-MC/2/38ada2a4cbb26fd15715680811ba8edd0

A point mutation of mitochondrial tRNA{\text{Leu(UUR)}} gene is responsible for a MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) subgroup of mitochondrial encephalomyopathies. In most cases, the mutant mitochondrial DNA (mtDNA) coexists with normal mtDNA in a heteroplasmic manner. In order to quantify the content of mutant mtDNA, we developed a quantitative method of PCR. Using this method, the distribution of the mutant mtDNA was examined in 32 different tissues among 18 autopsied organs from a patient with MELAS, who had shown hypophyseal dysfunction. The percentage of the mutant mtDNA at nucleotide number 3243 in each tissue was ranged between 22% and 95%. The content of the mutant mtDNA was at the highest (95%) in the hypophysis and higher in the cerebral cortex than in the white matter. This study shows a possible correlation of tissue dysfunction with accumulation of the mutant mtDNA within the brain.


http://www.sciencedirect.com/science/article/B6T06-44VX674-1/2/685e416f20487d3ca4a4df3e5190ffaa5

We have compared the induced expression of E-selectin in primary cultures of rat brain microvascular endothelial cells (EC), pericytes and in non-CNS microvascular endothelium stimulated with the cytokines, IL-1[\beta] (20 ng/ml), and tumor necrosis factor (TNF)-[\alpha] (75
ng/ml). Expression was studied at both the protein and mRNA levels. Fluorescence in-situ hybridization (FISH) was used to examine de novo synthesis of E-selectin mRNA. Laser cytometric analysis was used as a novel approach to the quantitation of FISH. In-situ hybridization was performed using two PCR-generated probes. The first probe (517 bp) spanned the lectin and epidermal growth factor (EGF)-like domain. The second probe (562 bp) spanned the CR3, 4, and 6 domains. E-selectin-specific mRNA was localized to the perinuclear regions of the EC. Both cytokines, IL-1[beta] and TNF-[alpha] significantly increased E-selectin gene expression in CNS EC but not pericytes. IL-1[beta] induced higher E-selectin mRNA levels than TNF-[alpha]. The maximum number of mRNA-positive cells was observed after stimulation for 4-6 h. Surface protein expression was sustained for up to 48 h following addition of cytokines. This was in contrast to the transient expression in non-CNS EC indicating that pure primary CNS EC display slightly different kinetics of E-selectin expression than non-CNS EC.


To clarify the role of apolipoprotein E (apoE) in the pathophysiology of Alzheimer's disease (AD), we used an RT-PCR method to investigate apoE and glial fibrillary acidic protein (GFAP) mRNA expression in the brain. ApoE mRNA was significantly more abundant in AD (0.379 +/- 0.191, mean +/- SD) than in control (0.125 +/- 0.073, p < 0.05) brain tissue, but in AD it was decreased in relation to the apoE-[epsi]4 gene dosage. The GFAP mRNA content also was greater in AD (5.96 +/- 2.94) than in control (3.80 +/- 2.78) tissue, and in AD showed an increase relative to the apoE-[epsi]4 gene dosage. AD patients who had long survival times showed high expression of apoE and low expression of GFAP. These results suggest that apoE suppresses the progression of AD, including gliosis, in the brain.


Two patients with amyloidosis caused by transthyretin (TTR) were investigated by immunohistopathologic, mass spectrometric, and molecular genetic methods. After confirming the immunoreactivity of TTR in the amyloid deposits using anti-TTR polyclonal antibody, a new method: centrifugal concentration and electrospray ionization mass spectrometry (ESI-MS) was employed to detect the variant TTR in the serum. Only 50 [mu]l of the serum and 30 [mu]l of the anti-TTR antibody were needed for the analysis. After incubation with the antibody, the samples were passed through a 1000 kDa cut off centrifugal concentrator to retain the antibody, thereafter, the filtrate was analyzed by ESI-MS. Several forms of normal and variant TTR were detected in the serum samples: unconjugated TTR, cysteine and cysteine-glycine conjugated TTR. In the patients, a variant form of TTR was detected with a 26.0 Da higher molecular weight than that of normal TTR. Single-strand conformation polymorphism (SSCP) and direct sequence analysis confirmed the presence of a one-base substitution situated at the codon 50 from AGT (Ser) to ATT (Ile) in both patients, that corresponded to the increased molecular weight of 26.0. The present diagnostic procedure demonstrates the usefulness of both ESI-MS and SSCP to screen for TTR related amyloidosis rapidly. Moreover, the DNA samples obtained from the band showing abnormal electrophoretic migration pattern in SSCP, facilitate the direct sequence analysis to
detect the unknown mutation, and the observed shift in molecular weight of the variant TTR in ESI-MS confirms the base substitution.


http://www.sciencedirect.com/science/article/B6T06-42G0KYK-6/2/5505caba443481a521b12146478e88258d

A novel mutation (Arg381Cys) in the second zinc-finger domain of early growth response 2 (EGR2) was identified in a late-onset Charcot-Marie-Tooth disease type 1 (CMT1) patient. This patient had initial symptoms of numbness and weakness in the leg at age 59, and a median nerve motor conduction velocity of 27 m/s. A sural nerve biopsy showed a severe loss of myelinated fibers with numerous onion bulbs. This is the first report of the EGR2 mutation presenting a late onset of CMT1 phenotype. Its mutation was a different amino acid substitution at codon 381 (Arg381His) which demonstrated congenital hypomyelinating neuropathy or early-onset CMT1. This report suggests that the EGR2 mutation represents divergent phenotypes at codon 381, which may be a mutation hotspot.


http://www.sciencedirect.com/science/article/B6T06-3RH634P-1/2/eee9e17eb503cd360f95eb8a3ec0fce2

To systematically elucidate the gene expression of inflammatory and immune modulators following middle cerebral artery occlusion (MCAO) in the rat, we studied interleukin-10 (IL-10) along with tumor necrosis factor alpha (TNF-[alpha]), interleukin-1 beta (IL-1[beta]) and interleukin-2 (IL-2). Gene expression of these cytokines was studied ipsilateral and contralateral to the MCAO, with mRNA expression levels evaluated 2, 4, 6, 8 and 12 h following permanent MCAO by reverse transcriptase polymerase chain reaction (RT-PCR). In the ischemic hemisphere TNF-[alpha] and IL-1[beta] mRNAs increased at 2 h following MCAO and peaked at 6 h, with IL-10 mRNA detected only at 6 h. Contralaterally, both TNF-[alpha] and IL-1[beta] mRNAs were expressed with a similar pattern to that in the ischemic hemisphere, but at lower levels, with no contralateral IL-10 expression. There was no difference in IL-2 gene expression between control and experimental animals in either hemisphere. These results demonstrate that IL-10 and TNF-[alpha], IL-1[beta] gene expression is induced early following MCAO. The temporal profile of these cytokines is similar to that seen in sepsis, where TNF-[alpha] induces IL-10; subsequently IL-10 inhibits TNF-[alpha] expression. The similarity of the temporal profile of cytokine expression in sepsis and cerebral ischemia suggests that IL-10 should be studied as a potential inhibitor of TNF-[alpha] production in ischemic brain tissue. The factors inducing contralateral expression of the inflammatory cytokines, TNF-[alpha] and IL-1[beta], along with the potential clinical significance of this remote cytokine gene expression, merit further study.

\textbf{Journal of the Society for Gynecologic Investigation} (10)

http://www.sciencedirect.com/science/article/B6T93-4CPJM90-6/2/b0a11eb5a1e58a7206ddd100dcf98624

ObjectiveTo investigate the possible role of human placenta in providing -serine to the developing fetus.

MethodsExpression of serine racemase in placenta was determined by reverse transcriptase polymerase chain reaction and northern analysis and confirmed by subsequent cloning. The transport of -serine by human ATB0 was characterized by expressing the cloned cDNA transiently in mammalian cells using the vaccinia virus expression system. -serine levels in maternal and fetal blood were measured by fluorescence high-performance liquid chromatography (HPLC) after derivatization of the amino acids with o-phthaldialdehyde and N-tertiary-butyloxycarbonyl--cysteine. Results mRNA for serine racemase was detected in placenta. ATB0 was capable of -serine transport, and the transport process is obligatorily dependent on sodium (Na+) with a Na+:substrate stoichiometry of 1:1 and saturable with a Michaelis-Menten constant of 310 +/- 30 [mu]M. Furthermore, studies have shown that ATB0 is not expressed in the maternal-facing brush border membrane of human placental syncytiotrophoblast. The circulating concentration of -serine in maternal serum is 5.8 +/- 0.5 [mu]M, and the corresponding value in the fetal serum is 14.6 +/- 1.2 [mu]M, indicating a two- to three-fold higher concentration of -serine in the fetus than in the mother. Conclusion We speculate that -serine is synthesized in human placenta by the racemization of -serine and that ATB0, expressed on the basal membrane of the syncytiotrophoblast, mediates the efflux of -serine into the fetal circulation in exchange for other amino acids in fetal blood.


http://www.sciencedirect.com/science/article/B6T93-3X9RY8X-3/2/b26a90041d2507854d0895b9f1500ada8

Objectives: Vascularity of the surface of the placenta in humans and of the placenta and fetal membranes in several species including sheep is an important determinant of intramembranous absorption of amniotic fluid. Our previous studies have shown that the total blood vessel surface area in ovine amnion and chorion increases with advancing gestation. Vascular endothelial growth factor (VEGF) is a potent angiogenic and permeability factor and is found to be expressed in the ovine placenta and fetal membranes. To investigate the role of VEGF in maintaining the absorptive function of the intramembranous microvessels, the present study was undertaken to determine the gestational change in gene expression of VEGF and its receptors, kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase (Flt-1), in ovine placenta, chorion, and amnion.

Methods: Total RNA was extracted from placental cotyledon, chorion, and amnion of ovine fetuses at 60-140 days of gestation. The relative abundance of VEGF, KDR, and Flt-1 mRNA was determined by Northern blot analysis, and VEGF molecular forms expressed were identified by reverse transcriptase polymerase chain reaction. The gestational changes in mRNA levels of VEGF and its receptors were analyzed by regression analysis. Results: In ovine placenta, chorion, and amnion, VEGF mRNA levels increased significantly from 60 to 140 days. The major VEGF molecular form expressed in these tissues was VEGF164, whereas VEGF120, VEGF144, and VEGF188 were present at lower levels. In the placenta, KDR was the primary VEGF receptor expressed, although Flt-1 was also detected at very low levels. In the amnion and chorion, KDR was the only receptor expressed. A gestational-dependent change in VEGF
receptor expression was not observed in the placenta and membranes. Conclusions: The increase in VEGF gene expression with advancing gestation in the amnion and chorion where KDR is expressed suggests that VEGF and its receptor are important determinants of vascularity and permeability, and thus exchange capacity, of the intramembranous pathway.


http://www.sciencedirect.com/science/article/B6T93-4B4NYRR-3/2/820a6f54409d49e7b1347ee364c8f544

Objective Three protease-activated receptor (PAR1, 3, and 4) isoforms have been shown to be responsible for the cellular effects of thrombin; another PAR isoform (PAR2) is responsible for the cellular effects of trypsin. The present studies sought to test the hypothesis that one (or more) of these PAR isoforms is expressed in myometrial tissue, thereby accounting for the uterotonic effects of these novel agonists. Methods The rat PAR3 and 4 isoforms were cloned from a rat spleen cDNA library. PAR isoform mRNA expression was determined by using reverse-transcriptase polymerase chain reactions (PCR) in Sprague-Dawley rats. Confirmation of the identity of the amplified mRNA was done by sequence analysis. Relative quantification of the PAR1 and PAR2 isoforms was performed using a real-time quantitative reverse transcriptase PCR (RT-PCR) technique. PAR protein expression was confirmed by Western blots using polyclonal antibodies. Results The rat PAR3 and 4 homologues showed significant sequence homology to the mouse and human amino acid and nucleotide sequences. The RT-PCR studies confirmed PAR1-4 expression in myometrium from rats in estrus. PAR3 was expressed in uterus, spleen, kidney, liver, lung, brain, and heart. PAR4 was expressed in uterus, spleen, and lung. Messenger RNA for the PAR1 and 2 isoforms was expressed during the second half of gestation in myometrium from timed-pregnant rats. In contrast, mRNA for the PAR3 and 4 isoforms was not detected in gestational myometrium. PAR protein expression appeared to match tissue mRNA expression patterns. Conclusion These RT-PCR studies confirmed ubiquitous expression of the PAR1 and PAR2 isoforms in myometrium and other rat tissues; in contrast, the PAR3 and PAR4 isoforms are expressed in a tissue-specific and gestationally related pattern.


http://www.sciencedirect.com/science/article/B6T93-4C8FYJY-5/2/a9660fe2ca4d608703128845a53ee9bf

Objective Despite emerging data on the in vitro modulatory effects of trophoblast-associated human leukocyte antigen G (HLA-G), its in vivo function needs to be determined. Immunohistochemical studies show a decrease in protein expression of trophoblast HLA-G in preeclampsia. Such a decrease in protein might be the consequence of a shift in HLA-G mRNA spliceform patterns. In an exploratory pilot study we determined trophoblast HLA-G mRNA spliceform distribution in preeclampsia. Methods Placental samples were collected immediately after cesarean delivery from pregnancies complicated by preeclampsia or the syndrome hemolysis, elevated liver enzymes, and low platelet count (HELLP) and uncomplicated normotensive pregnancies as controls. HLA-G mRNA spliceform distribution was analyzed using a semiquantitative reverse transcriptase polymerase chain reaction procedure. Results Analysis of HLA-G spliceform distribution showed a significant increase in frequency of the G5 form encoding for a soluble HLA-G molecule in preeclampsia. This increase in G5 form was not found in pregnancies complicated by HELLP. Conclusion The increased frequency in the expression of the
HLA-G G5 spliceform may play a role in the pathophysiology of preeclampsia, in particular through a recently suggested effect of this soluble HLA-G molecule on remodeling of the spiral arteries.


http://www.sciencedirect.com/science/article/B6T93-42XB4KM-8/2/765a065315245fd5e8cf50b3d38073e9

Objective: To determine whether aberrant expression of hormone receptor corepressors or coactivators or defects in estrogen receptor-mediated transcription might underlie resistance of ovarian cancers to hormonal therapy. Methods: Northern analysis, Western analysis, and polymerase chain reaction were used to examine expression of estrogen receptor (ER), progesterone receptor (PR), the nuclear receptor corepressors N-CoR and SMRT, and the steroid receptor coactivator BRG-1 in ovarian cancer cell lines and primary cancers. The effect of BRG-1 transfection on ER-mediated transcription was examined. We also determined the effect of estrogen and the pure estrogen antagonist ICI 182,780 on cell cycle profile and expression of ER. Finally, we examined the ability of estrogen to upregulate expression of known estrogen-responsive genes. Results: Among primary ovarian cancers, 18 of 52 (35%) expressed N-CoR, and 37 of 52 (71%) expressed SMRT, but there was no correlation between expression of corepressors and hormone receptor status. All of the primary ovarian cancers and cell lines expressed BRG-1. Estrogen stimulation of two cell lines expressing ER (SKOV3, OVCA 432) elicited low levels of ER-mediated transcription that was not enhanced by BRG-1 transfection. ICI 182,780 did not induce cell cycle arrest in these cell lines, but there was evidence of downregulation of ER, indicating a ligand-receptor interaction. However, estrogen did not elicit increased transcription of estrogen-responsive genes (PR, myc, fos, pS2). Conclusion: Inappropriate expression of the nuclear corepressors N-CoR and SMRT or the coactivator BRG-1 does not underlie the resistance of ovarian cancers to hormonal therapy. Further studies are needed to elucidate the mechanisms underlying the inability of ovarian cancers to undergo ER-mediated transcription if we hope to understand their resistance to hormonal therapy.


http://www.sciencedirect.com/science/article/B6T93-40J1DY9-3/2/a9971c6dce8ef9a3f0fb9dab5d82db9

Objective: To identify and localize the receptor(s) responsible for modulating vascular effects of corticotropin-releasing factor (CRF) during pregnancy. Methods: Reverse transcriptase-polymerase chain reaction (RT-PCR), competitive RT-PCR, and Western blot analyses were used to study the expression of CRF receptors (CRFR1, CRFR2[alpha], and CRFR2[beta]) in the aorta and uterine vascular bed of nonpregnant and late (day 18) and term pregnant (day 22) Sprague-Dawley rats. Immunohistochemistry was done to localize the CRF receptor in the aortic wall. There were six rats in each study group. Results: Only CRFR2[beta] was identified in the aorta and uterine vascular bed by RT-PCR and Western blot analyses. The PCR product was sequenced to confirm its identity. Competitive RT-PCR and Western blot analyses showed that expression of CRFR2[beta] is not different in late pregnancy compared with the nonpregnant but is decreased at term. Immunohistochemistry showed high expression of CRFR2[beta] on the aortic endothelial surface but low expression in the smooth-muscle layer. Conclusion: Only CRFR2[beta] is expressed in vasculature of nonpregnant and pregnant rats and may mediate the
vasorelaxant effect of CRF. This receptor is present predominantly in the vascular endothelium and to a lesser extent in the smooth muscle. The expression of CRF receptor in pregnant rat vasculature is down-regulated at term of gestation.


To determine whether serum-free (SF) conditioned media (CM) from several human breast cancer cell lines and primary stromal cell cultures contain factor(s) that mimic the marked stimulatory effects of serum on aromatase activity and aromatase P450 (P450arom) gene expression in adipose stromal cells in culture (ASC) in the presence of dexamethasone (DEX). Adipose stromal cells, harvested from fresh adipose specimens, were grown to confluence, switched to SF media, and then incubated in the presence of absence of DEX with CM from T47-D breast cancer cells, pre-treated with or without 17[beta]-estradiol (E2), and with CM from stromal cell cultures. Aromatase activity of the ASC was determined by the [3H]water release assay. Total RNA was isolated, and reverse transcription-polymerase chain reactions was performed to determine the expression of various 5'-termini. T47-D CM stimulated aromatase activity in a concentration-dependent manner, similar to that of serum, in ASC incubated with DEX. Estrogen potentiated this in a dose-dependent fashion. The ASC CM and endometrial stromal cell CM also markedly induced aromatase activity in ASC. Heat inactivation destroyed the stimulating ability of CM. The majority of P450arom 5'-termini expressed by ASC incubated with CM plus DEX contained the promoter I.4-specific sequence. Conditioned media from several breast cancer cell lines and primary stromal cell cultures can mimic the effects of serum in the presence of DEX to stimulate aromatase activity in ASC. These results suggest that undefined, heat-labile and proteinaceous factors are present in CM that stimulate P450arom expression in a fashion similar to that of serum.


Objective:To establish the role of phosphate and tensin homologue on chromosome 10 (PTEN) mutations in tumorogenesis of the ovary, we determined the mutational spectrum of the PTENgene in surgical specimens of ovarian carcinomas.Methods:The study group consisted of 86 ovarian cancer specimens (18 fluids, 68 solid specimens), including 30 primary ovarian cancer specimens and 56 of relapsed ovarian cancer from women with a median age of 57.9 years and a range of 27-85 years. Each of the nine exons of the PTEN gene was amplified separately by polymerase chain reaction (PCR). Both strands of the PCR products were sequenced directly by standard cycle sequencing procedures and subsequent computer-aided alignments with the wild-type sequence.Results:In ascitic fluids of two women with recurrence of cancer, we observed mutations: one seven-base-pair insertion at codon 52 (GATGATG) and the other a base-pair substitution resulting in an amino acid change (T131I). We found no mutation in the primary ovarian cancers.Conclusions:Our data indicate that PTEN mutations have a subordinate role in tumorigenesis of the ovary.

http://www.sciencedirect.com/science/article/B6T93-48BBJVM-5/2/ca59f0522fb73a70369bd0fbbc17d6e3

Objective To determine the impact of exogenous platelet-activating factor (PAF) on pregnancy outcome in the rat. Methods Carbamyl-PAF (0.05, 0.5, or 5.0 [mu]g/kg per hour) or vehicle was infused intravenously for 7 days by osmotic pump into timed pregnant rats. Infusion was begun on day 14 of a 22-day gestation. Maternal mean arterial blood pressures were measured on days 1, 4, and 7 of the infusion. On gestational day 21 (PAF infusion day 7), fetal and placental weights and viability were evaluated at hysterotomy. Uterine and placental PAF receptor expression was analyzed by reverse transcription-polymerase chain reaction and agarose gel electrophoresis. Data were analyzed by analysis of variance, [chi]2, or the Mann-Whitney U test as appropriate. Results Fetal weights were dose-dependently lower than control, by 19% and 35%, respectively, at dosages of 0.5 and 5.0 [mu]g/kg per hour (P < .05). Conclusions Exogenous PAF produces dose-dependent fetal growth restriction in the rat. Placental growth is particularly sensitive to PAF and, coupled with the dose-dependent decline in fetal growth, suggests a dose-dependent decline in function. An elevated level of PAF is detrimental to fetal growth and well-being in the rat.


http://www.sciencedirect.com/science/article/B6T93-4DN176B-1/2/1731415704f3a20f3780599fbd340ef

Objective We wanted to determine whether genetic variability in the gene encoding microsomal epoxide hydrolase (EPHX) contributes to individual differences in susceptibility to the occurrence of placental abruption. Methods The study involved 117 women with placental abruption and 115 healthy control pregnant women who were genotyped for two single nucleotide polymorphisms (SNPs), T-->C (Tyr113His) in exon 3 and A-->G (His139Arg) in exon 4, in the EPHX gene. Chi-square analysis was used to assess genotype and allele frequency differences between the women with placental abruption and the control group. In addition, single-point analysis was expanded to pair of loci haplotype analysis to examine the estimated haplotype frequencies of the two SNPs, of unknown phase, among the women with placental abruption and the control group. Estimated haplotype frequencies were assessed using the maximum-likelihood method, employing an expectation-maximization algorithm. Results Single-point allele and genotype distributions in exons 3 and 4 of the EPHX gene were not statistically different between the groups. However, in the haplotype estimation analysis we observed a significantly decreased frequency of haplotype C-A (His113-His139) among the placental abruption group compared with the control group (P = .007). The odds ratio for placental abruption associated with the low-activity haplotype C-A (His113-His139) was 0.552 (95% confidence interval, 0.358 to 0.851). Conclusions The use of two intragenic SNPs jointly in haplotype analysis of association demonstrated that the genetically determined low-activity haplotype C-A (His113-His139) was significantly less frequent in women with placental abruption.

Objectives The purpose of this study was to profile altered patterns of gene expression that characterize degenerative ascending thoracic aortic aneurysms and to compare these patterns with those observed for infrarenal abdominal aortic aneurysms. Methods Full-thickness aortic wall tissues were obtained during surgical repair of degenerative thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms (n = 4 each), with normal thoracic and abdominal aortas from organ transplant donors used as control preparations. Radiolabeled complementary DNA was prepared for each specimen and hybridized to complementary DNA microarrays, and differential levels of gene expression between aneurysmal and normal aortic tissues at each site were assessed by parametric statistics. Results Of 1185 genes examined, 112 (9.5%) were differentially expressed (P yes-1 oncogene, mitogen-activated protein kinase 9, and intercellular adhesion molecule 1/CD54). Results for 9 genes were independently confirmed by quantitative reverse transcriptase-polymerase chain reaction. Conclusions Thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms exhibit distinct patterns of gene expression relative to normal aorta from the same sites, with most alterations being unique to each disease. Degenerative aneurysms arising in different locations are thus characterized by a high degree of molecular heterogeneity, reflecting different pathophysiologic mechanisms.


Purpose Abdominal aortic aneurysm (AAA) is associated with chronic transmural inflammation and destruction of the elastic media. The purpose of this study was to elucidate molecular mechanisms that might orchestrate leukocyte recruitment into the outer aortic wall by determining whether CC chemokines contribute to development of aneurysm degeneration in an elastase-induced mouse model of AAA. Methods Adult male C57BL/6J mice underwent transient elastase perfusion of the abdominal aorta to induce development of AAA. At various intervals after elastase perfusion (0, 4, 7, 14 days), real-time reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assays were used to measure aortic wall expression of the CC ([beta]) chemokines, monocyte chemoattractant protein-1 (MCP-1) and regulated on
activation, normal T-cell expressed and secreted (RANTES). Expression of these chemokines by cultured mouse aortic smooth muscle cells (AoSMC) was similarly assessed after transient (5 minutes) exposure to elastase solutions in vitro. Results: Mouse aortic diameter (mean +/- SEM) increased to aneurysmal proportions by 14 days after elastase perfusion (from 0.51 +/- 0.03 mm to 1.34 +/- 0.32 mm; 163% increase; P P P 90-fold) of MCP-1 and RANTES, despite concomitant decrease in cell numbers. Conclusions: Increased mouse aortic wall expression of MCP-1 and RANTES occurs early in development of elastase-induced AAA and before onset of the chronic inflammatory response. Moreover, elastase directly stimulates AoSMC chemokine production in vitro. Elastase-induced medial SMC production of CC chemokines may therefore provide an important link between enzymatic injury, leukocyte recruitment, and aneurysmal degeneration of the aortic wall.


http://www.sciencedirect.com/science/article/B6WMJ-4CYY107-2C/2/010445b60f9b2fc0693172a98c072886

Purpose: Matrix metalloproteinases (MMPs) are considered to play a central role in the pathogenesis of abdominal aortic aneurysms (AAAs). Doxycycline (Dox) has direct MMP-inhibiting properties in vitro, and it effectively suppresses the development of elastase-induced AAAs in rodents. The purpose of this study was to determine if treatment with Dox suppresses AMPS within human aneurysm tissue and to elucidate the molecular mechanisms underlying this effect. Methods: Aneurysm tissues were obtained from 15 patients with an AAA, eight of whom had been treated with Dox before surgery (100 mg orally twice a day for 7 days). Protein extracts were examined by means of gelatin zymography and immunoblot analysis, and RNA was examined by means of reverse transcription-polymerase chain reaction (R-TPCR). The effects of Dox on MMP production were further examined in human THP1 mononuclear phagocytes in vitro. Results: No detectable difference was found between groups by using substrate zymography as a means of assessing total AMP activity, but Dox treatment was associated with a slight (24.4%) reduction in the activated fraction of 72-kDa gelatinase (MMP-2; P P P Conclusions: In addition to its recognized effects as a direct MMP antagonist, Dox may influence connective tissue degradation within human aneurysm tissue by reducing monocyte/macrophage expression of MMP-9 mRNA and by suppressing the post-translational processing (activation) of proMMP-2. Through this complementary combination of mechanisms, treatment with Dox may be a particularly effective strategy for achieving MAP inhibition in patients with an AAA.


http://jjco.oupjournals.org/cgi/content/abstract/32/7/266

Hereditary non-polyposis colorectal cancer (HNPPCC) is generally found from the patient's family history. The functional disorder of mismatch repair genes has been reported to be responsible for
HNPCC. The proband was a 28-year-old Japanese female who was admitted to our hospital with a diagnosis of descending colon cancer. Although there was no previous or family history of malignant disorders within the first- and second-degree relatives, the early onset of colon cancer prompted genetic analysis with suspicion of HNPCC. PCR analysis of the primary tumor showed DNA replication errors at the six microsatellite regions. PCR/direct sequential analysis of the peripheral lymphocytes revealed a germline frameshift mutation due to deletion of TTCAA at nt. position from 650 to 654 in exon 4 of the hMSH2 gene. According to the Human Mutation Database and International Collaborative Group on HNPCC Database, this type of the frameshift mutation is the first report in the hMSH2 gene.


http://jjco.oupjournals.org/cgi/content/abstract/35/3/158

The E-cadherin gene has been identified as having a physiological role in cellular attachment, and is hypothesized to participate in carcinogenesis. A polymorphism (an A to C substitution) in the 5'-untranslated region has a direct effect on E-cadherin gene transcriptional regulation. We explored the association between E-cadherin gene polymorphism and the risk of prostate cancer in a Japanese population. The subjects consisted of 236 patients with prostate cancer, 209 benign prostatic hyperplasia (BPH) patients and 139 male controls. A marginally significant difference was found between prostate cancer patients and male controls (P = 0.053). No significant difference was observed between prostate cancer and BPH patients. When patients with prostate cancer were divided into two groups, stage A+B and stage C+D, a significant difference was observed between progressive cancer patients (stage C+D) and male controls (odds ratio = 1.93, P = 0.016). It is possible that the presence of one A allele resulted in an increased risk of cancer progression.


http://jjco.oupjournals.org/cgi/content/abstract/32/1/3

Background: The expression level of human telomerase reverse transcriptase (hTERT) is correlated with telomerase activity and is expressed at high levels in malignant tumors. It is of interest whether expression of hTERT is regulated by methylation of the CpG island in the promoter of the hTERT gene. We examined hTERT expression and methylation status of the hTERT and other genes including p16. Methods: We analyzed methylation status by bisulfite treatment and polymerase chain reaction with single-strand conformation polymorphism analysis (PCR-SSCP) and expression of the hTERT by RT-PCR, in 13 cancer cell lines, eight white blood cell samples and 24 colorectal cancer tissues. Results: In the cancer cell lines, hTERT was expressed and the CpG island of the hTERT promoter was methylated. Most colorectal cancer tissues showed similar results. The promoter of hTERT was methylated in six cases, partially methylated in 17 cases and unmethylated in one case. All cases with methylation of hMLH1 or p16 also showed methylation of hTERT; however, some of the cases lacking p16 methylation also had hTERT methylation. Conclusion: Increased expression of hTERT is related to hypermethylation of hTERT in colorectal cancerous tissues as well as some cancer cell lines and discordant with hypermethylation of p16.

http://jjco.oupjournals.org/cgi/content/abstract/32/9/327

Background: Despite an increasing number of patients suffering from squamous cell carcinomas of the tongue, little is known about the molecular mechanisms involved in the origin and development of these neoplasms. Methods: We screened microdissected tongue squamous cell carcinoma (TSC) specimens from 28 consecutive, previously untreated, Japanese patients for mutations in the p53 tumor-suppressor gene single-strand conformation polymorphism analysis (exons 5, 6, 7, 8) and direct genomic sequencing. Results: Among them, 24 tumor specimens were well differentiated, three moderately and one poorly differentiated, according to the WHO classification. Mutations in the p53 tumor-suppressor gene were detected in only two out of the 28 (7%) tumor specimens. One was well differentiated and the other was poorly differentiated. Conclusions: Our results suggest that p53 gene mutations are less frequent in well differentiated TSC. These results indicate that mutations in the p53 gene may not be strongly involved in the development of well differentiated TSC.

Lebensmittel-Wissenschaft und-Technologie (1)


http://www.sciencedirect.com/science/article/B6WMV-4CPD5SX-1/2/189b1b8e5513319b0125a030d6c1c8b

In this study, a new identification method for Bifidobacterium species based on sequencing of a partial xylulose-5-phosphate/fructose-6-phosphate phosphoketolase gene (xfp) was evaluated. Approximately 500 bp sequences from 68 different strains including 34 type strains were compared. The results showed that this method discriminated between all bifidobacteria, generally with greater accuracy than 16S rDNA sequence analysis. It even clearly distinguished B. longum biovar infantis from B. longum biovar longum and B. longum biovar suis, as well as B. animalis from B. lactis.

Legal Medicine(13)


http://www.sciencedirect.com/science/article/B6W7W-4DFT078-3/2/c87af52a4262b65ab827a09ce3454f7
Twenty-six bone DNA identification cases are described. The postmortem periods of the studied remains ranged from three days to over 30 years, and the locations where the remains were found varied resulting in a variety of postmortem conditions. Nuclear DNA typing using an AmpFLSTR Profiler kit and mitochondrial DNA (mtDNA) typing of hypervariable regions 1 and 2 (HV1 and HV2) in a control region were performed both with decalcified and non-treated bone powder samples. Decalcification was shown to improve the success of DNA typing. The nucleotide sequences of the HV1 and HV2 regions were successfully determined in all cases examined. Nuclear DNA typing was very successful, more than half of the loci were typed during multiple amplifications (10 loci in one reaction) in 23 cases. Polymerase chain reaction (PCR) inhibition was observed in five cases including three samples that were found buried in soil. This inhibitory effect was identified as the result of unbalanced multiple PCR during the profiler test. These results revealed that DNA typing targeting nuclear DNA is a potentially powerful tool for bone identification.


http://www.sciencedirect.com/science/article/B6W7W-46NY92R-7/2/74250d16c167ccd263fe2064cdb49697

We have developed a new method for typing single nucleotide polymorphisms (SNPs) on the human Y chromosome based on a multiplexed single nucleotide primer extension. This method has the advantage that several SNPs are typed rapidly and simultaneously. We examined 15 different SNP loci on Y chromosome, M9, M105, M122, M125, M128, M130, SRY465, IMS-JST006241, IMS-JST006841, IMS-JST002611, IMS-JST003305, IMS-JST008425, IMS-JST021354, IMS-JST021355 and IMS-JST055457, in 159 Japanese males. From the typing results of these 15 loci, we found 13 haplotypes. Gene diversity for each locus ranged from 0.025 to 0.486 and the haplotype diversity was estimated to be 0.838. This method could be readily applied for personal identification and paternity testing.


http://www.sciencedirect.com/science/article/B6W7W-44CXRJJ-2/2/2aad436a6d7b1bce2371b397c4fa1ba1

Although an immunohistochemical investigation of pulmonary surfactant-associated protein A (SP-A) suggested a characteristic increase in fatal asphyxiation, no particular change was observed in the total amount of SP-A mRNA. SP-A is encoded by two highly similar genes, SP-A1 and SP-A2, which are differentially regulated in the expression. In the present study, to investigate the molecular pathology of SP-A, we established a method for quantitative RT-PCR assay of SP-A1 and SP-A2 mRNA transcripts. Using this method, fatalities from acute mechanical asphyxia (n=12) and drowning (n=9) were examined in comparison with control groups (n=17) of acute myocardial infarction (n=11) and peracute death due to brain lacerations (n=6). The SP-A1/A2 ratio (mean value) was markedly elevated in mechanical asphyxia (6.72) and drowning (5.64), whereas it was low in controls (acute myocardial infarction, 2.80; brain lacerations, 2.56). The analysis of the SP-A1/A2 ratio may assist interpretation of the molecular alterations of SP-A related to acute asphyxial death.
Inverse PCR technique was applied to type three major alleles (A1, B and O1) of the ABO blood group by simultaneously detecting separated allele-determining nucleotides (the 261st base in exon 6 and the 796th and 803rd nucleotides in exon 7) of the ABO gene. A sequence of about 1.7 kb from exons 6 to 7 of each allele was amplified, both termini of the fragment ligated, and allele-typing performed by the inverse PCR-restriction fragment length polymorphism (IP-RFLP) and allele-specific inverse-PCR (ASIP) methods. For intramolecular ligation, primers for the first PCR were designed to have Acc I-restriction sites within the sequences, and both termini of the 1.7-kb fragment were digested with Acc I. Using the IP-RFLP method, the inverse PCR product was digested with Kpn I, Nla III and Dde I, A1, B, O1-standard (OA) and O1-variant (OG) alleles were detected as 365-bp, 272-bp, 193-bp and 128-bp fragments, respectively. By the ASIP method using four allele-specific primers, 222-bp, 124-bp and 232-bp fragments were amplified from A1, B and O1 templates, respectively. These techniques would be applicable to detecting separated polymorphic regions of some other genes.

We report a case with the inconsistency that the red blood cells lacked both A- and B-antigens while the serum showed reactivity with control B-red cells but not with A-red cells. A- and B-antigens were examined by serological blood typing and immunohistochemical staining, and DNA analyses were performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), DNA sequencing, and hot-stop PCR. A-antigens were demonstrable in the nail of the subject by serological study and immunostaining. DNA analyses showed that the nail retained a small amount of A-allele comparing to that of O-allele. Those genomic analyses of ABO genes were useful for demonstration of A allele in the nail of an individual with the absence of A antigen on red blood cells and the corresponding antibody in serum.

The allele-specific inverse polymerase chain reaction (PCR) technique, which has been explored to detect two linked polymorphic regions simultaneously, was applied to genotype the Se system. The major alleles of the Se system in Japanese are Se, sej defined by a single nucleotide substitution in the Se allele, and sefus generated by recombination between the Sec1 and FUT2 genes. The first PCR products using gene-specific primers were self-ligated, and each allele was detected by the second inverse-PCR using allele-specific primers. The 340, 331 and 353-bp products were finally amplified from Se, sej and sefus templates, respectively. The first PCR products without mung bean nuclease treatment were not self-ligated and non-specific fragments were amplified in the second PCR, suggesting that non-templated adenylation occurred at the termini during the first PCR. Nuclease digestion of the first PCR products that blunts their termini
was found to reduce interference of non-templated adenylation with the intramolecular ligation and to improve the genotyping markedly. This modified allele-specific inverse-PCR method is applicable to analyze haplotypes consisting of separated single nucleotide polymorphisms and recombinant genes.


http://www.sciencedirect.com/science/article/B6W7W-44G8CDV-37/2/c0849594b3bd4abba78e9f99d49a28be

The ABO phenotype of a bloodstain (B) on a knife that was used as a weapon in an attempted murder case was found to be different from that of the Peruvian victim's blood (AB). Serological analysis showed that the A-antigenicity was much weaker than B antigenicity, suggesting that the victim's phenotype was A2B or A3B. So, the ABO genotypes of the knife bloodstain and the victim's blood were determined by DNA analysis. The 261st G deletion, specific to the O1 allele, was not detected in the specimens by restriction fragment length polymorphism analysis. Also, the 871st A, specific to the A3 allele, was not found by the allele-specific amplification method. Amplified product length polymorphism and direct sequencing methods finally demonstrated that the typical B sequence was found in one allele and a single C deletion in the 1,059th-1,061st C stretch in the other allele, indicating that the ABO phenotype of the bloodstain and victim's blood were A2B.


http://www.sciencedirect.com/science/article/B6W7W-46NY92R-5/2/c500ad24184da7d34998b0624088fcdf

The polymorphism of the Sec2 gene, which determines Se blood type, has been reported. This study presents an Se genotyping system by the allele-specific polymerase chain reaction amplification method. The Se, sej and sefus alleles were amplified using allele-specific primers. The Sec1, Sec2 and sefus genes were analyzed by DNA sequencing. The 299-bp Se, 146-bp sej and/or 312-bp sefus allele-specific products were amplified and detected in the native polyacrylamide gel. The 314th-316th nucleotides of the Sec1 gene were CCC, which were different from the nucleotides GGG reported previously by Kelly et al. [J Biol Chem 270 (1995) 4640]. This Se genotyping system is a simple method available for the forensic science field in Japan. The crossover region of the sefus gene is a 164-bp stretch corresponding to the regions between the 253rd and 416th of the Sec1 gene and between the 211th and 374th of the Sec2 gene.


A multiplex PCR system for five Y-STRs (DYS441, DYS442, DYS443, DYS444 and DYS445) has been improved to increase the probability of obtaining a DNA typing result from aged samples. Newly designed PCR primers for amplification of the DYS441 and DYS442 loci and optimization
of PCR conditions enabled successful typing from blood and semen stains that had been stored for more than seven years at room temperature. Analysis of 340 Japanese males revealed 7, 5, 6, 5 and 4 alleles at the DYS441, DYS442, DYS443, DYS444 and DYS445 loci, respectively, yielding 122 haplotypes with a cumulative haplotype diversity of 0.97.


http://www.sciencedirect.com/science/article/B6W7W-47PG72T-V/2/b078826e1f8ff1c4773883cc890098dd

This study investigated the identification of unidentified bodies through HLA DNA typing of aortic tissues. Eight aortas were collected at autopsy from six bodies found in water, one burnt body, and one mummified body, respectively. DNA was extracted from 10-20 mg of aortic tissue with a PUREGENE(R) DNA Isolation Kit and phenol/chloroform. HLA (A, B, DR or DQ locus) alleles were typed using the hot start polymerase chain reaction-sequence specific primers (PCR-SSP) method with a Dynal AllSet+(TM) SSP "low resolution" kit for each locus. The aorta was still retained in degraded samples, those from the drowned, the burnt, and the mummified bodies. In each case, approximately 0.04-3.84 [mu]g of DNA was recovered from 1 mg of fixed tissue. We typed 8/8 for the DR, 4/4 for the DQ, 3/3 for the A, and 3/3 for the B locus from the samples. Based on these results and the finding that DNA extraction is easier from the aorta than from other samples such as bone or tooth, we considered this method to be useful for forensic samples.


http://www.sciencedirect.com/science/article/B6W7W-44B1Y7K-2/2/7a11b97f5f09f6bf92139ccfcde7eebe9

Designing of PCR tests for the RHC allele is difficult because of the high DNA sequence homology between RHC and RHD genes, which differ by only a one-nucleotide substitution at position 48 in exon 1 of the RHCE gene. We sequenced the promoter region of the RHCE gene, and compared our results with the reported sequence. Genomic DNA was prepared from blood samples collected from 656 Japanese donors. The DNA segment encompassing the promoter region and exon 1 of the RHCE gene from 30 donors was amplified by PCR and analyzed by DNA sequencing. Four nucleotide differences between RHC/c and RHD were found at positions -468, -304, -58, and -46. On the basis of the nucleotide differences at positions -468 (RHCE vs. RHD) and -292 (RHC vs. Rhc), we then developed a novel polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for RHC/c genotyping. Analysis of the genomic DNA from the 656 donors revealed that this method could discriminate RHC from Rhc, irrespective of the RHD genotype, with only a few exceptions. The combination of our system and the intron 2-based PCR-RFLP method previously reported may prove to be more accurate than either of the methods alone, and therefore, useful and valuable for RHC/c genotyping.

We performed multiplex polymerase chain reaction (PCR) for the TH01, TPOX, CSF1PO, and vWA loci using a newly designed pair of primers that yield smaller fragments than reported previously [Fujii et al., J Hum Genet 45 (2000) 303; Lederer et al., Int J Legal Med 114 (2000) 87]. These loci can be detected in the range of 74-143 bp amplifying products. This system required genomic DNA in a range of 80 pg to 2 ng, and proved to be a sensitive typing method. We compared our system against the GenePrint Fluorescent STR Multiplex Systems CTTv (Promega, Madison, WI, USA), using DNA extracted from old bloodstains left to stand for 17-26 years at room temperature. With our designed system, all allele-typing efforts were successful in the range of 1-5 ng DNA, while no signal peaks were detected, even with when using 10 ng of DNA GenePrint Fluorescent STR Multiplex Systems CTTv.


This study confirms the presence of a novel variable number of tandem repeats polymorphism, designated as HumDN1, in intron 4 of the human deoxyribonuclease I (DNase I) gene. Genotyping was performed without difficulty by PCR-amplification and separation by agarose gel electrophoresis in 423 Japanese, originating from four geographically diverse areas in Japan, and 89 Germans. The HumDN1 allele variability was due to different numbers of 56-bp repeat sequences, and five different alleles were distinguished with apparent size between 364 and 588 bp. Although there was a general uniformity for the polymorphism in the Japanese population, significant differences in genotype distribution were found between the Japanese and German populations. Furthermore, linkage disequilibrium between the HumDN1 and DNase I protein polymorphisms was revealed.

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K-562 cells were cultured in HL-60 cell growth-conditioned medium (GCM) for up to 96 h. Myeloperoxidase (MPO) mRNA was transiently detected by reverse transcription-polymerase chain reaction (RT-PCR) techniques at 12, 24, and 48 h. The de novo expression of MPO protein was subsequently detectable by intracellular flow cytometry at 24, 48, 72 and 96 h. Immunogold staining and cytochemical analysis demonstrated granularly-sequestered MPO in approximately 40% of HL-60 GCM-cultured cells after 48 h of culture. The sequential detection of MPO mRNA and MPO biosynthesis is considered an indicator of serial maturation evocative of myeloblastic cells, and suggest that K-562 cells maintain the ability to differentiate along this lineage.

http://www.sciencedirect.com/science/article/B6T98-4BY4PSJS/2/40bba214793b8b2f88a7a0b2ec272542

We studied 98 female patients in remission (2-240 months) from childhood ALL to determine the clonality status of their hematopoiesis. Thirty-one (31.6%) were heterozygous at the PGK locus for the BstX1 endonuclease restriction site, permitting X-linked clonality assays to be performed. Two patients were in relapse at the time of study and were excluded. We used the PGK-PCR clonality assay (PPCA) to analyze DNA from PMN and mononuclear cells of the remaining 29 female patients. All (29/29) patients demonstrated polyclonal hematopoiesis. These data show that remission from childhood ALL involves reestablishment of polyclonally derived hematopoiesis in all patients studied.


http://www.sciencedirect.com/science/article/B6T98-49J8T38-5/2/d448bddef37302cc24b2f6fc4e107973

Background: We analysed the methylation status of a panel of 10 genes including p15, p16, DAPK, p73, VHL, E-CAD, MGMT, RAR[beta], RIZ1, and ER. Methods: The gene promoter methylation status was studied by methylation-specific polymerase chain reaction (MSP) with primers for methylated (M-MSP) and unmethylated (U-MSP) DNA in the bone marrow of 13 patients with myeloma, and one patient with plasmacytoma. Result: None of the 10 genes tested were methylated in eight normal bone marrow samples. For the positive control, the sensitivity of M-MSP ranged from 1 x 10-2 for E-CAD and MGMT, to 1 x 10-4 for p73. Of the eight diagnostic myeloma marrow samples, hypermethylation of p15, p16, E-CAD, DAPK and ER occurred in six (75%), four (50%), seven (87.5%), eight (100%), and six (75%) patients. Similarly, of the five samples from patients who progressed from plateau phase, hypermethylation of p15, p16, E-CAD, DAPK, and ER occurred in five (80%), two (40%), five (100%), five (100%), and three (60%). None of the cases had hypermethylation of RIZ1, p73, VHL, RAR[beta], and MGMT. At diagnosis, all patients had concurrent hypermethylation of at least three genes, and five (62%) had concurrent methylation of four or more genes. One patient with plasmacytoma had methylation of E-CAD, ER, and DAPK. Conclusion: p15, p16, ER, DAPK, and E-CAD (but not RAR[beta], p73, VHL, RIZ1, and MGMT) were frequently methylated in MM at both diagnosis and disease progression. Future studies of larger scale are needed to identify the genes responsible for disease progression.


http://www.sciencedirect.com/science/article/B6T98-47GHMRS-1/2/c9c5178d5b0a0e95caef2fe167764065

TAL1 disruption at 1p32 [del(1p)] is a common rearrangement in the development of T-cell acute lymphocytic leukemia (T-ALL). The del(1p) are usually interstitial 90 kb deletions placing TAL1
under control of the SCL interrupting locus (SIL) gene forming the SIL-TAL1 fusion product. A reverse transcriptase real-time PCR assay to quantify SIL-TAL1 fusion genes is described. A SIL-TAL1 fusion gene RNA transcript was built that permitted absolute standard curves to be generated. Sensitivity of the RT-PCR assay was determined to be 10 cells (CEM cell line) in 106 human lymphocytes. Peripheral blood lymphocytes from 10 healthy adults and 10 neonates were assayed. None of the samples showed any SIL-TAL1 expression. However, when lymphocytes from three adults were cultured in vitro the SIL-TAL1 transcript was detectable in the RNA isolates. No RAG2 expression was detected in these expanded samples, suggesting that the clones bearing the SIL-TAL1 fusion gene may have existed at low levels prior to the ex vivo expansion.


A cytogenetic and N-ras point mutation study was done in patients with primary myelodysplastic syndrome (MDS) from Rio de Janeiro, Brazil, in order to evaluate the progression of preleukemic states to overt leukemia. Cytogenetic analysis was performed in 50 patients with MDS and clonal chromosomal abnormalities were detected in 19 (38%) of them. Patients with refractory anemia (RA) or with ringed sideroblasts (RARS) presented normal karyotypes or single abnormalities as del(5q) or -Y, while patients in more advanced states as RA with excess of blasts (RAEB), RAEB in transformation (RAEB-t) and chronic myelomonocytic leukemia (CMML) showed complex karyotypes and single abnormalities involving chromosomes 7 or 8, which were related to poor prognosis and elevated risk of transformation to acute myeloid leukemia (AML). The frequency of ras activation was studied in these 50 patients with MDS. Samples of bone marrow were screened for oncogenic point mutations by DNA amplification followed by oligonucleotide hybridization analysis (PCR-ASO) at codon 12 of N-ras proto-oncogene. We detected N-ras point mutations in 21 patients (42%). Progression from MDS to AML was observed in 9 patients (18%). The correlation analysis between N-ras point mutations and specific chromosomal abnormalities indicated that although mutated N-ras was found in cells with del(5q) and monosomy 7, cells with those abnormalities and normal N-ras were also identified. Otherwise trisomy of chromosome 8 showed a correlation with N-ras point mutations and in all cases, patients showed progression of MDS to AML during the follow-up study. MDS comprises a heterogeneous group of hematopoietic disorders and probably several steps are implicated in the evolution to AML. In this work we suggest that one possible pathway of leukemogenesis in MDS includes N-ras point mutations in association with trisomy of chromosome 8.


Amifostine is a phosphorylated aminothiol that has besides anti-oxidative and cytoprotective properties, also survival- and growth-promoting effects on hematopoietic progenitor cells. Clinical studies have demonstrated that infusions with amifostine are able to increase erythro-, myelo-, and thrombopoiesis in some patients with myelodysplastic syndromes (MDS). Since clonal and non-clonal progenitors can coexist in early phase MDS, we have studied if amifostine exerts a selective growth-promoting effect on clonal or non-clonal cells. For this purpose, purified CD34+
marrow progenitors from nine female MDS patients were grown in short- and long-term cultures. Clonality was studied on individual colonies using polymorphisms in the human androgen receptor assay (HUMARA) locus. Three patients had growth of residual non-clonal progenitors at baseline. Continuous exposure to 100 nM amifostine exerted a growth-promoting effect on progenitors in 50% of the patients. HUMARA patterns of individual colony-forming unit granulocyte macrophage (CFU-GM; 5/9) and 5 week long-term culture-initiating cells (LTC-IC; 2/9) were compared without and with amifostine exposure. We did not observe preferential stimulation of clonal or non-clonal progenitors. Based on these results, the stimulation of committed and immature progenitor growth in MDS by amifostine, is non-selective and does not favor nor suppress the growth of residual non-clonal cells.


http://www.sciencedirect.com/science/article/B6T98-3VXHRT-2/2/2dd731b53de8a77a0a3e24ae6c9c4daf

We investigated the possible influence of recombinant (r) sIL-6R on the growth of three IL-6 non-responsive or weakly IL-6 responsive long-term myeloma cell lines. The three cell lines chosen for the study (U266, L363 and Fravel) all expressed gp130 but differed in their expression of IL-6R and IL-6. mRNA analysis by northern blot and reverse transcriptase polymerase reaction showed that the cell line U266 was the only one that expressed IL-6 mRNA. Only U266 and L363 expressed IL-6R mRNA. 125I-rIL-6 binding studies and FACS analysis, using biotinylated IL-6 and antibodies directed against the IL-6R and gp130, showed corresponding results on the protein level. Addition of rsIL-6R resulted in induction of IL-6 responsiveness in L363 cells, whereas the 3H-thymidine incorporation of the cell lines U266 and Fravel was unaffected by rsIL-6R addition. In conclusion, the IL-6 unresponsive growth of several long-term myeloma cell lines in vitro can in some, but not all cases, be due to a deficiency in exogenous sIL-6R.


http://www.sciencedirect.com/science/article/B6T98-40378GB-7/2/5c0820c31098d0aaa34f5d17ff9fcc52

Normal myeloid cells of monocytic and granulocytic origin express the metallopeptidase cluster of differentiation 13 (CD13) on the surface just as leukemic blasts in most acute myeloid leukemias (AML). A minor percentage of AML patients, however, lack the surface expression of CD13 antigen. To study this difference in CD13 surface expression, specific CD13 mRNA from 44 individuals were quantified by competitive reverse transcription polymerase chain reaction (RT-PCR). Absolute values for CD13 transcripts were normalised against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript levels to control for variations in sample preparation and mRNA degradation. By correlating normalised CD13 transcript levels and CD13 surface expression, a subgroup of AML patients was identified, having simultaneous diminished levels of myeloid CD13 transcripts and surface expression of the corresponding antigen. For this subgroup we suggest CD13/aminopeptidase N (APN) gene expression to be restricted primarily by limited amounts of transcripts. For the majority of AML patients determinants in addition to transcript levels must be involved in regulating CD13/APN gene expression.


Mantle cell lymphoma (MCL) is an aggressive malignancy and new treatment modalities must be established to increase patient survival time. In the search for new therapeutic targets, reliable and well-characterised in vitro models are essential. In this study, we have characterised three MCL cell lines (SP53, Granta 519 and NCEB1) in comparison with primary tumours from MCL, follicular lymphomas (FL), a FL cell line (RL), a Burkitt lymphoma cell line (RAJI) and five different B cell populations from healthy individuals. Expression profiling was used to determine the relative expression of >12000 transcripts in these samples, and flow cytometry analysis was performed to establish a phenotypic signature for each of the cell lines. In addition, the cell lines were sequenced, and the frequency of somatic mutations and immunoglobulin (Ig) variable heavy chain (VH) usage were determined. We show by hierarchical clustering that the cell lines retain a genetic signature similar to primary MCL, which readily separated the MCL samples from the other lymphoma cell lines and the FL tumours. Furthermore, the MCL cell lines showed differences in the frequency of VH somatic mutations (0-2.1%). The increased number of mutations in NCEB1, compared to the other MCL cell lines, was in agreement with a decreased expression of CD31, CD44, CXCR5, CCR7 and CCR6. Taken together, our data show that the cell lines are clearly derived from MCL tumours and expressed similar genetic and phenotypic signatures compared to primary tumours, which confirmed their usefulness as in vitro models.


http://www.sciencedirect.com/science/article/B6T98-3RGSWRB-J/2/c2ac4410559a3c8c2f9d738f43de5a26

Several means of analyzing minimal residual disease (MRD) in leukemia involving the rearranged T cell receptor (TCR) gene have been described. We investigated MRD in leukemia with TCR [beta] rearrangement by examining TCR [beta]-chain RNA. A complementary DNA (cDNA) corresponding to the variable region of the TCR [beta]-chains originating from the peripheral blood or bone marrow from four patients was amplified. Single strand conformation polymorphism (SSCP) analysis of amplified cDNA showed that all four patients had monoclonal leukemia with TCR [beta] rearrangement; two patients had V[beta]2+ leukemia, another patient had V[beta]14+ leukemia and the other had V[beta]9+ leukemia. Flow cytometry supported this finding. Sequencing of the V[beta]2-complementarity determining region 3 (CDR3), V[beta]9-CDR3 and V[beta]14-CDR3 revealed monoclonality. To investigate MRD using TCR [beta]-chain RNA, cDNA from each patient was diluted with the cDNA of a healthy person and amplified using a specific CDR3 clonotype primer. A band in the ethidium bromide-stained agarose gel was detected from samples diluted 10 000-fold. SSCP analysis determined which V region gene was utilized in monoclonal leukemic cells. The leukemic cell specific TCR, determined in such a manner, may be a target for immunotherapy. Because the MRD of T cell malignancy can be easily examined once the CDR3 clonotype primer is made, this novel analysis is considered to be a useful method.

A number of transcription factors (TFs) have been reported that play crucial roles in hematopoiesis. However, only little is known about how these factors are involved in the mechanisms of hematopoietic development and lineage commitment. To investigate the roles of TFs in human B-cell precursors (BCPs), the present study analyzed the expression of the following 16 hematopoietic TFs: AML1, C/EBP[alpha], C/EBP[beta], C/EBP[gamma], C/EBP[epsilon], E2A, Ets-1, GATA-1, GATA-2, GATA-3, Ikaros, IRF-1, Pax5, PU.1, T-bet and TCF-1 in 30 human BCP-leukemia cell lines. All BCP-leukemia cell lines were found to be positive for the expression of AML1, C/EBP[gamma], E2A, Ets-1, IRF-1, Pax5 and PU.1 at the mRNA level. The mRNA expression of C/EBP[alpha], C/EBP[beta], C/EBP[epsilon], GATA-2, Ikaros, T-bet and TCF-1 was detected in 2 to 29 of the cell lines. Eight BCP-cell lines showed positivity for the dominant negative Ikaros isoform Ik6, while others were positive for expression of Ik1, 2, 3 and 4. GATA-1 and GATA-3 were universally negative. The expression of C/EBP[alpha], PU.1 and T-bet was positive at the protein level in five, 29 and four out of 30 BCP-cell lines, respectively. Cell lines were stimulated with interleukin (IL)-7 and/or interferon (IFN)-[gamma] to investigate the regulation of TF expression. T-bet was clearly induced in the two cell lines NALM-19 and NALM-29 after stimulation. C/EBP[beta] and IRF-1 were up-regulated in both cell lines and TCF-1 was down-regulated in NALM-19. No significant changes were observed for the other 12 TFs. The present report could provide useful information in the study of the role of TFs on normal and malignant human BCPs.


http://www.sciencedirect.com/science/article/B6T98-4BP9WBF-MV/2/a7288214b97a21de39a5c77e2bed4b2d

By combining allele-specific PCR amplification and a PCR-based quantitation approach, a method has been developed to estimate the mutated K-ras gene content in the blood of AML patients as a percentage of total K-ras. One PCR primer set was designed not to discriminate between mutant K-ras and wild-type K-ras and thus amplified the total K-ras gene. The other PCR primer set was designed to be allele-specific for K-ras genes containing a G to C mutation at codon 12. This primer set could discriminate the mutant and wild-type genes when the proportion of the mutated sequence was 0.2% of the total K-ras gene. To test the method on biological specimens, genomic DNA samples were analyzed from the peripheral blood of a patient who had secondary AML with the same codon 12 K-ras mutation. Two samples taken from this patient 2 months apart during follow-up had myeloblast cell contents of 67 and 80%. However, the percentage of mutated K-ras was 50% in both samples, suggesting that this patient may be inherently heterozygotic in this particular mutation. This ratio of mutated to normal K-ras in the patient's cells was confirmed by RNA-SSCP analysis and RNA sequencing. This quantitation method can provide a sensitive and specific estimation of the content of mutated K-ras alleles in patient samples.


http://www.sciencedirect.com/science/article/B6T98-3WM54SD-7/2/b6c8713c0156735b57abd18936a82790
Determination of the MDR-phenotype in patients suffering from AML is an important hallmark of treatment outcome but is often complicated by technical problems in P-gp assessment. A PCR-MIMIC strategy was employed to construct PCR-fragments for a competitive and quantitative mdr1 reverse transcription-PCR-assay. Using K562 cells, which had been selected for drug resistance to the epipodophyllotoxin VP16, a stepwise increase of mdr1 levels depending on the concentration of VP 16 was shown with the MIMIC technique. Comparison of mdr1 levels in drug selected K562 cells with the corresponding levels for P-gp and functional data indicated a mRNA threshold that has to be exceeded for the full expression of the MDR-phenotype. Subsequently mdr1 levels of 34 samples of de novo acute myeloid leukemia were determined with the PCR-MIMIC strategy. Ten patient samples could be identified with elevated mdr1 levels which were substantially lower than the levels observed in the MDR-cell line K 562 0.7 [mu]M VP16. Outcome analysis revealed that eight of the ten patients had an unfavourable prognosis and did not achieve CR after induction chemotherapy. Coexpression of mdr1 and CD 34 was not associated with CR in all examined cases. Moreover all these patients had unfavourable cytogenetic aberrations. These data indicate a sensitive technique with applicability in patient material.


http://www.sciencedirect.com/science/article/B6T98-3YB565M-3R/2/22f2bee714bcb9d4d290d932ca16e54

The eradication of minimal residual blast populations by activation of autologous cytotoxic cells with interleukin 2 (IL-2) is a new promising tool in the treatment of acute myelocytic leukemia (AML). However, the immunological effector cells are not yet clearly defined. The present study was designed to investigate the presence of cytotoxic precursor cells in active AML and to identify phenotypical and functional characteristics of autologous anti-leukemic cytotoxic effector cells. For this purpose, mononuclear cells (MNC) containing at least 70% leukemic blasts were isolated from bone marrow of untreated AML and cultured in the presence of 3000 IU/ml recombinant IL-2 (rIL-2) for 6-8 weeks. Under these conditions, T-cells were selected in the bone marrow cultures and overgrew the leukemic blasts. The resulting T-cell populations were cloned by limiting dilution and the clones obtained were characterized for their phenotypical and functional patterns. Totally, cloning resulted in 68 clones and a few cell lines. The clonality was verified by RT PCR analysis of TCR V[beta] gene expression. All clones obtained stained positive for CD2, CD3, DR and CD56. The vast majority (68%) of T-cell clones/lines was CD4+, a few clones expressed CD8 (19%) or CD4 and CD8, and four clones were of TCR[gamma][delta] origin. Seven of 15 clones tested, including three CD4+, two CD8+ and two TCR[gamma][delta]+-clones were found to be cytotoxic against autologous leukemic blast cells. All except one clone expressed oncolytic activities against allogeneic blasts too. One of the TCR[gamma][delta]+-clones demonstrated NK activity by lysis of K562 targets. The majority of the T-cell-clones released IL-2, IL-8, TNF-[alpha], GM-CSF but only a few IFN[gamma] and expressed high levels of mRNA for IL-2, TGF-[beta] and IL-10. None of the clones was found to produce IL-3, IL-4, IL-7 and TNF-[beta]. The data provide evidence of the existence of T-cell precursors in untreated AML bone marrow differentiating to cytotoxic cells with activity against autologous and allogeneic AML blast cells.


http://www.sciencedirect.com/science/article/B6T98-433NP9N-G/2/7cf951a8077be0a8f660f02ed4267058
Mastocytosis is a term used for a group of disorders characterized by abnormal growth and accumulation of tissue mast cells (MC) in one or more organ systems. In patients with systemic mastocytosis (SM) the clinical course may be indolent or aggressive or even complicated by leukemic progression or an associated clonal hematologic non mast cell lineage disease (AHNMD). However, at first presentation (diagnosis) it may be difficult to define the category of disease and the prognosis. We report on a 48-year-old female patient with SM with urticaria pigmentosa-like skin lesions and mediator-related symptoms. She was found to have splenomegaly, a high infiltration grade (MC) in bone marrow biopsies (>30%), mild anemia, and a high serum tryptase level (>500 ng/ml). In addition, she exhibited discrete histologic signs of myeloproliferation in the 'non-affected' marrow and monoclonal blood cells established by C-KIT 2468A -> T mutation (Asp-816-Val) -analysis and HUMARA assay. Despite these findings, however, the clinical course was stable over years and no AHNMD or organ impairment developed. Because of the 'intermediate' clinical signs and absence of progression to aggressive disease, we proposed the term 'smouldering mastocytosis'.


http://www.sciencedirect.com/science/article/B6T98-3W258FG-8/2/41d24b57616471a39726f20dae6ae01f

Deoxycytidine kinase (dCyd kinase) is important for the phosphorylation of several different nucleoside antimetabolites. To understand the significance of dCyd kinase levels in chemotherapy, dCyd kinase mRNA levels were evaluated in several cells with a quantitative competitive polymerase chain reaction (PCR) assay. dCyd kinase catalytic activity and intracellular ara-CTP production were also compared with the levels of dCyd kinase mRNA. The assay was able to show: (i) that dCyd kinase catalytic activity and dCyd kinase mRNA levels were correlated in cells; (ii) that dCyd kinase mRNA levels were more sensitive in lower levels of 10 amol/[mu]g of total RNA; and (iii) in cytosine arabinoside (ara-C)-resistant cells, both dCyd kinase mRNA levels and intracellular ara-CTP levels were lower compared with levels in sensitive cells. The PCR assay for dCyd kinase mRNA could be useful in the selection and monitoring of patients treated with nucleosides that are activated by this enzyme.


Six patients received an allogeneic bone marrow transplant from HLA-identical ABO-mismatched donors. ABO genotype of erythroid burst-forming units (BFU-E) from peripheral blood was analyzed using polymerase chain reaction with sequence specific primers (PCR-SSP). After bone marrow transplantation (BMT), engraftment of donor cells by ABO genotypic analysis of BFU-E was compared with ABO phenotypic analysis of red blood cells (RBCs). During the early stage after BMT, ABO genotype of BFU-E in the recipients converted to that of the donors. In contrast, mixed ABO phenotype of RBCs persisted for about 3 months. In one patient, autologous hemopoietic cell recovery was detected by the ABO genotypic analysis before clinical manifestation. ABO genotypic analysis of BFU-E is relevant for engraftment after ABO-mismatched BMT.


We studied Cyclin D1 (CyD1) and CD23 mRNA expression with real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) method. CyD1 expression in peripheral blood of seven mantle cell lymphoma (MCL) patients was found to be 1305.4 times higher than in 24 B-chronic lymphocytic leukemia (CLL) patients. CD23 expression in CLL was found to be 54.8 times higher than in MCL. These differences were statistically significant, and no overlap was found in CyD1 expression intensities between MCL and CLL. RQ-PCR allows rapid, simple and accurate quantification of CyD1 and CD23 expression, even from small samples, and is thus useful for the diagnosis of MCL and CLL.


http://www.sciencedirect.com/science/article/B6T98-4C118PD-T/2/d2befeea1057c3d44522c4688537835

The level of cytosolic thymidine kinase (TK1) mRNA in lymphocytes from six healthy people and in lymphocytes from five patients with untreated chronic lymphatic leukemia (CLL) was determined with competitive polymerase chain reaction (competitive PCR). Using this procedure we have shown that in patients with CLL, there is an overexpression of TK1 mRNA without corresponding enzymatic activity. The TK1 mRNA level is approximately 100-fold higher in lymphocytes from CLL patients than in lymphocytes from healthy persons. A high level of TK1 mRNA without corresponding enzyme activity may indicate a defect in the processing of the enzyme. This may disturb the cells' normal feedback system and thereby influence the development of malignant conditions.


http://www.sciencedirect.com/science/article/B6T98-452RBCH-7/2/7d00300d5750cdaba78f48d78f718258

As a part of our continuing efforts to develop gene therapy for acute myelogenous leukemia (AML), this study was undertaken to evaluate the possibility of using autologous bone marrow stromal fibroblasts (BMSFs) as a target cell population. Autologous BMSFs in AML were isolated from the stromal layers of long-term bone marrow culture (LTBMC) using immunomagnetic beads. BMSFs exhibited rapid proliferation even in the absence of growth factors. Cultures stimulated with bFGF produced significantly increased numbers of BMSFs than cultures without added growth factors. Using LNC/LacZ retroviral vector, the transduction efficiency of BMSFs was 13+/-4% at a 5 multiplicity of infection (MOI). LNC/interleukin-2 (IL-2)-transduced BMSFs produced between 1200 and 4800 pg of IL-2/106 cells per 24 h. Using adenoviral vector Adv/LacZ, the transduction efficiency was 84+/-10% at 100, and 92+/-8% at a MOI of 1000. Although the addition of basic fibroblast growth factor, epidermal growth factor, or platelet-derived growth factor did not affect the transduction efficiency, they increased the numbers of transduced
cells significantly (P6 cells per 24 h. Our finding that the genetically engineered autologous BMSFs of AML could be successfully established in vitro implies that BMSFs obtained from LTBMC might be considered as a target cell population for certain types of clinical gene therapy in AML.


http://www.sciencedirect.com/science/article/B6T98-3RGT92H-2/2/68fb30d0061f3dc42b5a8141be4b4e16

All-trans-retinoic acid (ATRA) has been used as a potent therapeutic agent to induce differentiation of acute promyelocytic leukemia (APL) cells, and granulocyte colony-stimulating factor (G-CSF) has been reported to enhance this effect of ATRA in vitro. We investigated the effects of ATRA and three myeloid growth factors, including G-CSF, on the growth of the leukemic stem cells of 10 APL patients. G-CSF was the most powerful stimulator of leukemic colony formation in five out of 10 patients, but was neither the major stimulant of self-renewal of the blast stem cells nor an inducer of maturation. In contrast, ATRA was highly effective in inducing morphological maturation of leukemic promyelocytes, but variable results were obtained in regard to its effects on the growth of blast stem cells: ATRA suppressed both clonal growth and self-renewal in some patients, but was inactive or even had stimulating effects in the other patients. Similar variable effects were observed with the combination of ATRA and G-CSF. These findings indicate that the differentiation-inducing effect of ATRA is not always associated with growth inhibition of leukemic stem cells in vitro and justify the use of chemotherapy in conjunction with ATRA in the treatment of APL.


http://www.sciencedirect.com/science/article/B6T98-4B5JM6V-6/2/1136f69233c135c369191f6d1b166f915

Survivin, a member of the inhibitor of apoptosis protein (IAP) gene family, has been detected widely in fetal tissue and in a variety of human malignancies. In the current study, we investigated the expression of IAP family proteins in bone marrow samples from acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL) and control cases by quantitative real-time RT-PCR method and an immunohistochemical approach. Overexpression of survivin and cIAP2 mRNA was significant in CLL bone marrow cells (P<0.05, respectively) compared with control samples. By immunohistochemistry, survivin was detected in a few scattered myeloid cells in all cases of control bone marrow. Concerning the ALL bone marrow, more than half the cases demonstrated positive expression of survivin (8 out of 13), while the majority of CLL cases (20 out of 21) exhibited intense expression of survivin. The differential subcellular localization of survivin was distinct between ALL and CLL cases. ALL cells essentially revealed nuclear localization of survivin as well as cytoplasmic signals in some cases, while CLL cells from the majority of cases predominantly showed cytoplasmic expression. Next, RT-PCR was performed for the expression of survivin and its splicing variant, survivin-2B and survivin-[Delta]Ex3 in ALL and CLL cells, as the distribution of these variants would be regulated by nuclear/cytoplasmic transport system. In both ALL and CLL bone marrow samples, the expression of wild-type survivin was more predominant than that of survivin-2B or survivin-[Delta]Ex3, although the expression of survivin-[Delta]Ex3 was prominent in samples from survivin-expressing ALL cases. Thus, the splicing of
survivin mRNA may be differently regulated in ALL and CLL cells, causing distinct manners of nuclear/cytoplasmic transport of survivin protein. In conclusion, our observations indicate a differential regulatory mechanism for the expression of IAP family proteins in ALL and CLL cells, although the functions of IAP families and the mechanisms of nuclear/cytoplasmic transport of survivin should be clarified in future studies.


http://www.sciencedirect.com/science/article/B6T98-47K2J0N-2/2/4311d691880f917ee6e25676ee094f0

Cancer/testis antigens (CTA) are an expanding family of immunogenic proteins selectively expressed in human neoplasms. As little is known about the expression of serologically identified CTA in leukemias so far, we investigated the expression of 5 CT genes (SSX-1, HOM-MEL-40/SSX-2, HOM-TES-14/SCP-1, SCP-3 and NY-ESO-1) in leukemic blood samples obtained from patients with either acute lymphatic leukemias (ALL) or myelocytic leukemia (AML). RT-PCR-analyses showed no expression of any of the CT-genes in the leukemia samples of 19 patients with AML, whereas frequent expression was found in ALL. In the 17 ALL cases studied, SCP3a, SSX-1, HOM-MEL-40/SSX-2 and HOM-TES-14/SCP-1 were expressed in 47, 29, 29 and 12%, respectively, whereas no case was positive for NY-ESO-1. 65% of patients with ALL showed expression of at least one, 41% of two or more of the five CT-genes investigated. We conclude that a majority of the ALLs might be amenable for specific immunotherapeutic interventions. However, the identification of additional antigens with a frequent expression in leukemias is warranted to allow the development of widely applicable polyvalent leukemia vaccines.


http://www.sciencedirect.com/science/article/B6T98-47YH3V0-7/2/0c199dfe3517c8bbce8105056d0971133

STAT5 phosphorylation has been noted in 69-95% of AML cases by Western blotting. We used flow cytometry to measure phosphorylated STAT5 on a semi-quantitative scale. The method was validated on K562 cells, which constitutively express phosphorylated STAT5, but lose this when BCR-abl tyrosine kinase activity is blocked by STI571. Phosphorylated STAT5 was found to measure 2.22+/−0.09 relative fluorescence units (RFU) falling to 0.925+/−0.005 RFU in the presence of STI571. Phosphorylated STAT5 expression was 0.99 to 2.09 RFU in 28 primary AML samples. There was no logical cut-off point between positive and negative fluorescence. FLT3 internal tandem duplications, found in 11/28 samples, were not significantly associated with the level of phosphorylated STAT5 expression. We conclude that STAT5 phosphorylation can be measured sensitively by flow cytometry in AML and that its expression should not be dichotomised as present or absent.

Expression of the c-myb proto-oncogene is developmentally regulated at the level of transcription elongation. In pre-B cells, complete c-myb transcripts are produced, whereas transcripts are attenuated near or within a 300-base pair (bp) interval of the first c-myb intron in mature cells. Hypothesizing that transcription attenuation results from a protein complex that physically impedes the progress of RNA polymerase II through the intron, we used electrophoretic mobility shift assays (EMSA) to search for DNA-binding activities that correlated with downregulation of c-myb transcription. We identified a stage-specific DNA binding activity, termed ABF, present in mature B cells but not in pre-B cells. ABF binds to a 15-bp DNA element located within a 300-bp BstEII-XbaI fragment. DMSO-treatment of murine erythroleukemia cells results in rapid downregulation of c-myb transcription and upregulation of ABF DNA binding activity. Thus, ABF binding activity correlates with downregulation of c-myb transcription in two systems. Preliminary biochemical characterization of ABF from mature B cells demonstrates that its primary DNA-binding component is a 64-kDa-protein. We hypothesize that this factor may represent a member of the transcriptional attenuation complex.


In solid cancers, defective DNA mismatch repair (MMR) is most commonly caused by hMSH2 or hMLH1 mutations, or epigenetic silencing of hMLH1 by promoter hypermethylation, and results in the acquisition of characteristic frameshift microsatellite mutations of mononucleotide repeats located within the coding regions of defined target genes. We previously identified hMSH2 mutations in T-cell lymphoblastic lymphoma (T-LBL) patient tumor samples and others have reported coding region microsatellite mutations in T-cell acute lymphoblastic leukemia (T-ALL) cell lines. Thus, while MMR gene mutations are known to occur in some human T-lymphoblastic tumors in vivo, it is still unknown if the coding region microsatellite mutations detected in human cell lines also occur in vivo or if hMLH1 or hMSH2 promoter hypermethylation contributes to defective MMR in these tumors. We analyzed the TGF[beta]RII (A)10 and caspase-5 (A)10 coding region repeats in 16 human T-LBL/ALL patient tumor samples and identified six with microsatellite mutations in one or both repeats. There was no evidence of hMSH2 or hMLH1 promoter methylation as assessed by standard methylation specific PCR or by a novel temperature gradient electrophoresis (TTGE) method that analyzed 25 and 30 CpG sites in the hMLH1 and hMSH2 promoters, respectively. Our results indicate that coding region microsatellite mutations characteristic of defective MMR occur in some human T-LBL/ALL in vivo but not as a consequence of hMLH1 or hMSH2 promoter hypermethylation. Furthermore, the identification of TGF[beta]RII and caspase-5 coding region mutations in vivo implicates these genes in the pathogenesis of human T-LBL/ALL.

The c-kit mutation Asp-816->Val is detectable not only in neoplastic mast cells (MCs) in patients with systemic mastocytosis (SM) but also in most associated hematologic non-MC lineage disease (AHNMD). In order to prove a monoclonal disease evolution we investigated DNA of pooled microdissected single cells for the presence of the mutation in a patient with SM and concomitant chronic myelomonocytic leukemia (CMML). LightCycler melting curve analysis and direct sequencing of nested polymerase chain reaction (PCR) products revealed the c-kit mutation in tryptase-positive MC and in leukemic CD15-positive cells in bone marrow infiltrates, but not in colonic epithelial cells, thus, suggesting a monoclonal evolution of SM and concurrent CMML on the basis of a somatic mutation in a common hematologic progenitor.


http://www.sciencedirect.com/science/article/B6T98-3VWP0J1-10/2/f36f4b109d79d2e8544506a86ea45e4e

We report the establishment and characterization of two cell lines, MEC1 and MEC2, that grew spontaneously on two subsequent occasions from the peripheral blood (PB) of a patient with B-chronic lymphocytic leukemia (B-CLL) in prolymphocytoid transformation. The patient was EBV-seropositive, his leukemic cells were EBNA negative, but the spontaneously grown cell lines are EBNA-2 positive. In liquid culture MEC1 cells grow adherent to the vessel wall and as tiny clumps; MEC2 cells do not adhere and form large clumps. The doubling time of MEC1 is 40h and of MEC2 is 31h. Both cell lines express the same light ([kappa]) and heavy chains ([mu], [delta]) as the fresh parental B-CLL cells at the same high intensity, share the expression of mature B cell markers (CD19, CD20, CD21, CD22), differ in the expression of CD23 and FMC7, are CD11a+, CD18+, CD44+, CD49d+, CD54+ and express at high levels both CD80 and CD86. CD5 is negative on MEC1 cells (as on the vast majority of parental cells) and it has been lost by MEC2 cells after several months of culture. The cells have a complex karyotype. The tumour origin of MEC1 and MEC2 has been demonstrated by Southern blot analysis of the IgH loci and by Ig gene DNA sequencing. They use the VH4 Ig family and have not undergone somatic mutations (94.8% homology with germline Ig gene 4-59). Cytofluorographic analysis and RT-PCR reveal that MEC1 and MEC2 overexpress Bc1-2 together with Bax, express large amounts of Bcl-xL and trace amounts of Bcl-xS.


http://www.sciencedirect.com/science/article/B6T98-461XG0N-4/2/dab009b9623e60e262c6385c8f94042f

CD22, one of the important markers for diagnosing B-lineage acute leukemia, was expressed in mature basophil granulocytes. We then investigated the expression of CD22 and other B cell- and basophil-related molecules in 25 human acute leukemia cell lines to find the phenotype of the virtual common progenitor of B and myeloid lineage. Surface and cytoplasmic expressions of antigens were analyzed using a flow cytometer and an essential antibody panel used for diagnosing acute leukemia as well as cytokine receptors and basophil-related enzymes. Messenger RNA expression of Fc[epsiv]R1 and CD22 was also analyzed. Peroxidase-positive and -negative myeloid leukemias showed eosinophil- and basophil-type expression of enzymes, respectively. Early myeloid and B-lineage cells expressed basically similar combinations of cytokine receptors and various combinations of mRNA listed above, while T-lineage cells did not. The virtual common progenitor of B and myeloid lineage cells may be defined as immature cells simultaneously expressing B and basophil phenotypes.

http://www.sciencedirect.com/science/article/B6T98-44J720S-5/2/b4e49ab605ef5bc6cf990a9619272c2e

P-glycoprotein (P-gp)/multi-drug resistance 1 (MDR1) gene is recognized to be, at least in part, responsible for the refractoriness to chemotherapy of leukemia. The transcriptional mechanism of MDR1 gene is largely unknown. However, recent reports have clarified that early growth response 1 gene (Egr1) positively regulates MDR1 transcription, while Wilms' tumor suppressor gene (WT1) does negative regulation of MDR1 gene expression in 12-O-tetradecanoylphorbol-13-acetate treated K562 cells. In addition, Egr1 and WT1 are structurally related transcription factors and bind to quite similar DNA sequences. Our study of mRNA expression profile of Egr1, WT1 and MDR1 in fresh AML samples demonstrated that there are disease-specific patterns. Egr1 mRNA was frequently and strongly expressed in monocytic leukemia cells, especially in AML M4 cells. WT1 mRNA was undetectable in t(8;21) AML cells. mRNA expression of MDR1 was frequent in AML M1 and t(8;21) AML cells, in which the expression level was highest in AML M1 and was low in monocytic leukemia (M4 and M5). Then, expression level of MDR1 was inversely correlated with Egr1. By liquid culture of leukemia cell lines and fresh AML cells with the addition of all-trans retinoic acid (ATRA), modulation of P-gp/MDR1 and Egr1 was observed and the pattern of modulation was divided into four groups: (1) blastic AML type, in which distinct expression of P-gp/MDR1 and CD34 was not influenced by ATRA; (2) t(8;21)AML type, in which P-gp/MDR1 expression was augmented by ATRA, while CD34 was kept high; (3) AML M3 type, in which P-gp/MDR1 expression was reduced with granulocytic differentiation by ATRA; (4) monocytic AML type, in which P-gp/MDR1 expression was augmented by ATRA, while CD34 expression decreased, and strong Egr1 expression was downregulated just prior to the augmentation of P-gp/MDR1 expression. WT1 expression was not influenced by the addition of ATRA in each group. Previous reports have suggested that P-gp/MDR1 plays an important role in resistance to chemotherapy, and is recognized as one of the stem cell marker. However, P-gp/MDR1 expression augmented by ATRA, which was observed in monocytic AML, was recognized as a functional molecule of mature monocyte/macrophage, because CD34 expression decreased and CD13 expression increased by ATRA. Finally, expression of P-gp/MDR1 in monocytic leukemia, which was functionally confirmed by Rh123 efflux study, was thought to be closely related to the characteristic modulation of Egr1 expression by ATRA.


http://www.sciencedirect.com/science/article/B6T98-48XCCH3-2/2/a9f4ee6a1ed1b17866109bae76c4e75

Expression of cell cycle-regulating genes was studied in human myeloid leukemia cell lines ML-1, ML-2 and ML-3 during induction of differentiation in vitro. Myelomonocytic differentiation was induced by phorbol ester (12-o-Tetradecanoyl-phorbol-13-acetate, TPA), tumor necrosis factor [alpha] (TNF[alpha]) or interferon [gamma] (INF[gamma]), or their combination. Differentiation (with the exception of TNF[alpha] alone) was accompanied by inhibition of DNA synthesis and cell cycle arrest. Inhibition of proliferation was associated with a decrease in the expression of cdc25A and cdc25B, cdk6 and Ki-67 genes, and with increased p21Waf1/Cip1 gene expression, as measured by comparative RT-PCR. Expression of the following genes was not changed after induction of differentiation: cyclin A1, cyclin D3, cyclin E1 and p27Kip1. Surprisingly, cyclin D1 expression was upregulated after induction by TPA, TNF[alpha] with INF[gamma] or BA. Cyclin D2 was upregulated only after induction by BA. The results of the expression of the tested genes
obtained by comparative RT-PCR were confirmed by quantitative real-time (RQ) RT-PCR and Western blotting. Quantitative RT-PCR showed as much as a 288-fold increase of cyclin D1 specific mRNA after a 24 h induction by TPA. The upregulation of cyclin D1 in differentiating cells seems to be compensated by the upregulation of p21Waf1/Cip1. These results, besides others, point to a strong correlation between the expression of cyclin D1 and p21Waf1/Cip1 on the one hand and differentiation on the other hand in human myeloid leukemic cells and reflect a rather complicated network regulating proliferation and differentiation of leukemic cells.


We measured telomerase activity (TA) in bone marrow samples from 214 patients with CLL and correlated it with patients’ characteristics and survival. In >50% of cases (126/214; 59%) no detectable TA was found. There was no difference in TA between previously treated (n=153) and untreated (n=61) patients (P=0.4), or patients with various Rai (0-IV) stages (P=0.85). TA correlated significantly with white blood cell and lymphocyte count (P=0.02 and 0.01, respectively) but not with bone marrow cellularity, [beta]2-microglobulin ([beta]2M), or other patient characteristics. Patients who had no TA had slightly lower [beta]2M and lower lymphocyte counts (P=0.5 and 0.04, respectively) as compared with patients with detectable TA. However, there was no correlation between TA and survival. This data suggests that TA may not play a significant role in the clinical behavior of CLL.


Nasal-type natural killer/T-cell lymphoma (NKL) is a rare but distinct malignancy that often involves the mid-facial region and the gastrointestinal tract. This study is the first genome-wide allelotyping analysis on this rare lymphoma. We applied 382 microsatellite markers covering loci which spanned 22 autosomes to screen for allelic imbalances (AI) in six intestinal NKL. The most common chromosomal regions of allelic imbalances were found in 11p, 9q and 13q. Novel spots of allelic losses spots found at 2p21, 2q37.22, 18p11.21 and 18q12.1. In spite of presence of a few recurrent loci of imbalances, the allelotyping results show that NKL is heterogeneous.


From 5% to 20% of patients with agnogenic myeloid metaplasia (AMM) will evolve into a terminal leukemic phase; N-RAS gene mutations are the most common gene abnormalities detected in patients with leukemia. The present study was designed to see if N-RAS gene mutations are associated with the leukemic transformation in AMM. Over a 9 year period, in a single institution,
43 patients with AMM were studied. Of these, ten patients were found to be in leukemic phase. The results showed that none of the patients in chronic phase (40 patients) had N-RAS gene mutations, while two patients in leukemic phase showed this gene mutation. One patient was found to have a codon 12 mutation with arginine substituting for glycine (GGT→CGT); the other was a codon 12 mutation with glutamine substituting for glycine (GGT→GAT). The present study suggests that N-RAS mutations are rare events in the chronic phase of AMM, and are only occasionally found when patients have evolved into leukemic transformation. Further studies to search for other gene abnormalities in AMM may be warranted.


http://www.sciencedirect.com/science/article/B6T98-4D48XF7-1/2/17a581d689f0dea6e71df5c0b4a167c9

Cytokines play a key role in the differentiation, growth and survival of hematopoietic cells in the bone marrow (BM) stroma microenvironment. The mechanisms by which stromal derangements may contribute to the evolution of hematopoietic neoplasias are largely unknown. Here, we characterized BM stromal cells isolated from children with acute lymphoblastic leukemia and determined the effect of the interaction between stromal cells and lymphoblasts on cytokine expression as well as the effect of prednisolone using mono- and co-culture models. The analyses demonstrate that (1) stromal cells and lymphoblasts display different patterns of cytokine gene expression individually. (2) Stromal cells influence gene expression of cytokines in lymphoblasts and vice versa. (3) Glucocorticoid substitution inhibit cytokine gene expression in stromal cells. These findings indicate that stromal cells are important components involved in malignant hematopoiesis and also in response to therapy.


http://www.sciencedirect.com/science/article/B6T98-4CJV8YV-7/2/e2860d193f75b9263669da1ab8a82086

Bone marrow cells of patients with myelodysplastic syndromes (MDS) frequently undergo apoptosis, though the apoptotic cell ratio decreases when overt leukemia (OL) develops. Thus, we compared the expression of the inhibitor of apoptosis protein (IAP) gene family proteins in bone marrow samples from control, MDS, OL transformed from MDS (MDS -> OL), and de novo acute myelogenous leukemia (AML) subjects by the quantitative real-time RT-PCR method and an immunohistochemical approach. Overexpression of mRNA for survivin, cIAP1, NAIP and XIAP was significant in MDS bone marrow cells compared with control samples. However, the expression of mRNA for survivin, cIAP1 and cIAP2 exhibited a remarkable decrease after the development of OL (MDS -> OL). By immunohistochemistry, survivin was found to localize to the nucleus of myeloid cells in the majority of MDS cases. Next, the chronological changes in the expression of IAPs were determined in cases of MDS with evolution of OL. Although the expression of cIAP1 and cIAP2 revealed a sudden or gradual decrease as OL developed, survivin in many cases and XIAP in the majority of cases exhibited a peak of expression before a decline, indicating that these IAPs could be associated with the early events in the development of OL.


A real-time quantitative-polymerase chain reaction (RQ-PCR) targeting the immunoglobulin heavy chain (IgH) gene has been used for the quantification of minimal residual disease (MRD) in B-cell hematological malignancies. In non-Hodgkin lymphoma (NHL), experimental costs are increased, as a large number of primer-probe sets are required because of diversity, due to somatic and ongoing mutations of the IgH gene. We developed an allele-specific oligonucleotide (ASO) combined with a germline consensus probe-based RQ-PCR assay and examined MRD in peripheral blood stem cells (PBSC). The IgH consensus probes were adapted in seven (50%) of 14 amplifiable cases. Patients with heavily contaminating tumor cells in PBSC relapsed after PBSC transplantation. Our strategy will contribute to the development of a cost-efficient, precisely quantitative and systemic detection assay for MRD in NHL.

Yokohama, A., M. Karasawa, et al. (2001). "Molecular detection of tumor cells at diagnosis invading the bone marrow and peripheral blood of patients with aggressive or indolent lymphomas." *Leukemia Research* 25(9): 749.

http://www.sciencedirect.com/science/article/B6T98-4599B2H-3/2/a15755c7fc0c884b564b67fcd7c90479

We studied tumor cell invasions of bone marrow and peripheral blood in patients with various types of advanced non-Hodgkin's lymphoma by amplifying complementarity determining region III using the polymerase chain reaction (PCR) method and developing patient-specific probes. After molecular engineering, we could detect tumor cells in bone marrow from seven of 11 cases and in peripheral blood from six of 11 cases, despite negative results in four cases studied morphologically. Indolent cases were more likely to yield positive results than aggressive cases. The reason may be different biological behaviors among the histological types.


http://www.sciencedirect.com/science/article/B6T98-45CX73K-D/2/628b2ca821241d47ba9852010e2a9eb6

We developed a real-time RT-PCR assay for the quantification of topoisomerase II (topo II) mRNA level. It was applied on peripheral leukaemic cells from 23 patients with acute myelogenous leukaemia (AML) and 23 with chronic lymphocytic leukaemia (CLL). RNA template dilutions from 0.25 to 25 ng per reaction were used as standard curves for topo II[alpha], [beta] and the internal control 18S rRNA. About 57% (26/46) and 26% (12/46) of the specimens had detectable topo II[alpha] and [beta] mRNA, respectively. The correlation between these two factors was [rho]=0.7 and P=0.0001. No relationship between topo II[alpha] or [beta] mRNA level and response to chemotherapy was found in AML patients (n=19 assessable for response). Our method is rapid and convenient for quantification of topo II[alpha] and [beta] mRNA levels, and could be suitable for investigation in a larger population.
Fetal malnutrition is associated with development of impaired glucose tolerance, diabetes and hypertension in later life in humans and several mammalian species. The mechanisms that underlie this phenomenon of fetal programming are unknown. We hypothesize that adverse effects in utero and early life may influence the basal expression levels of certain genes such that they are re-set with long-term consequences for the organism. An excellent candidate mechanism for this re-setting process is DNA methylation, since post-natal methylation patterns are largely established in utero. We have sought to test this hypothesis by investigating the glucokinase gene (Gck) in rat offspring programmed using a maternal low protein diet model (MLP). Northern blot reveals that fasting levels of Gck expression are reduced after programming, although this distinction disappears after feeding. Bisulphite sequencing of the hepatic Gck promoter indicates a complete absence of methylation at the 12 CpG sites studied in controls and MLP animals. Non-expressing cardiac tissue also showed no DNA methylation in this region, whereas brain and all fetal tissues were fully methylated. These findings are not consistent with the hypothesis that programming results from differential methylation of Gck. However, it remains possible that programming may influence methylation patterns in Gck at a distance from the promoter, or in genes encoding factors that regulate basal Gck expression.

It has been suggested that dopamine might play a role in the regulation of the immune system. In this study, reverse transcriptase-polymerase chain reaction (RT-PCR) was used to investigate the expression of mRNA for the different subtypes of dopamine receptors in the rat lymphocytes. D1, D3 and D5 receptor mRNAs were identified. These results provide further evidence for the interaction of dopamine systems and the immune system, and suggest to further investigate whether the immunosuppressive actions of dopamine and dopaminergic drugs might depend on a direct interaction with dopamine receptors on the lymphocyte membrane. Moreover, they suggest the suitability of this animal species to further investigate the correlation between changes in the expression of central and peripheral dopamine receptors produced by manipulations of the dopamine systems.
We investigated the effects of chronic cocaine exposure on the microcirculation in the rat mesenteric venules under both non-inflammatory and FMLP-induced inflammatory conditions. Chronic cocaine significantly increased WBC rolling flux in both conditions, and potentiated FMLP-induced leukocyte-endothelial cell adhesion (LEA). In cocaine-treated animals, total WBC number increased by 91%, and the ratio of white blood cell to red blood cell velocity was significantly lower, while vessel diameter was unchanged. Chronic cocaine decreased serum levels of tumor necrosis factor alpha (TNF-\[alpha\]) and interleukin-6 (IL-6), but had no effect on interleukin-1 beta (IL-1[\beta]). Expression of intercellular adhesion molecule-1 (ICAM-1) was increased in mesenteric venules following chronic cocaine exposure, and may be one of the mechanisms underlying enhancement of FMLP-induced LEA. The increase in WBC count, WBC flux and LEA, and the change in cell velocity seen in the cocaine-treated animals could cause a decrease in effective vessel diameter and a change in intravascular resistance, and may underlie the progressive vascular damage seen in chronic cocaine-abusing individuals.


The identity of the serotonin (5-HT) receptor(s) that mediate(s) contraction in canine coronary artery and saphenous vein remains controversial. Ring segments of endothelium-denuded coronary artery and helical strips of saphenous vein were suspended in organ chambers for measurement of isometric force. 5-HT, [alpha]Me-5-HT and sumatriptan contracted both coronary artery and saphenous vein and the non-selective 5-HT receptor antagonist 1-naphthylpiperazine (100nM) blocked 5-HT-and sumatriptan-induced contraction in both tissues. The agonist rank order potency for contraction (5-HT>sumatriptan>[alpha]Me-5-HT>5-MeOT>5-MeT) was similar in both tissues and was consistent with that for a 5-HT1D receptor. Oligonucleotide primers specific for the 5-HT1D receptor sequence were designed for use in a polymerase chain reaction (PCR). cDNA derived from total RNA or mRNA from canine tissues was used in the PCR. PCR resulted in the amplification of a 632 base pair sequence in both canine coronary artery and saphenous vein; consistent with that expected for the 5-HT1D receptor. Southern blot analysis, with an oligonucleotide probe internal to the sequence amplified by the PCR primers, confirmed that the sequence amplified by PCR was the 5-HT1D receptor. Thus, the 5-HT1D receptor is expressed in canine coronary artery and saphenous vein and taken together with the pharmacological data, supports the possibility that a 5-HT1D-like receptor mediates contraction in these two tissues.


Dopamine D2 receptor (D2-receptor) expression and its coupling to Gi sensitive adenylate cyclase was investigated in human neuroblastoma SHSY-5Y cells. Incubation of SHSY-5Y cells in the presence of 100 nM retinoic acid (RA) for 24 hours resulted in phenotypic differentiation accompanied by a 47% increase in D2-receptor mRNA and a significant increase in the specific binding of a D2-receptor antagonist, [3H]YM09151-2. Stimulation of D2-receptors in differentiated
cells by LY171-555, a D2-agonist, attenuated cellular cAMP levels by 30%. The effect of LY171-555 on cAMP levels was blocked by the D2-antagonist, (-)sulpride. Application of these drugs to control undifferentiated cells or differentiated cells incubated with vehicle only had no effect on cellular cAMP levels. These studies suggest that differentiated SHSY-5Y cells express functional D2-receptors and will provide a useful model for future studies on the regulation of expression and function of D2-receptors in cellular differentiation of neuronal cells.


http://www.sciencedirect.com/science/article/B6T99-47X0WMJ-1/2/bd4a235633f1c232fb845e187c9bc057

We previously showed that lymphocytes possess the necessary components to constitute an independent, non-neuronal cholinergic system; these include acetylcholine (ACh) itself, choline acetyltransferase (the ACh-synthesizing enzyme), and both muscarinic and nicotinic ACh receptors (AChRs). In addition, we showed that stimulation of AChRs with their respective agonists elicits a variety of biochemical and functional effects, suggesting that lymphocytic cholinergic system is involved in the regulation of immune function. In nerve terminals, choline taken up via the high-affinity choline transporter (CHT1) is exclusively utilized for ACh synthesis. In the present study, therefore, we investigated the expression of CHT1 in T-lymphocytes. Reverse transcription-polymerase chain reaction analysis revealed that MOLT-3 cells, a human leukemic T-cell line used as a T-lymphocyte model, expressed CHT1 mRNA, but that the CEM and Jurkat T-cell lines did not. Consistent with that finding, specific binding of [3H]hemicholinium-3 (HC-3), an inhibitor of CHT1, and HC-3-sensitive [3H]choline uptake were also detected in MOLT-3 cells. These results suggest that CHT1 plays a role in mediating choline uptake in T-lymphocytes and provides further evidence for the presence of an independent lymphocytic cholinergic system.


http://www.sciencedirect.com/science/article/B6T99-3YYTH72-41/2/8c8e01ec8bc3030febce7238e62e5249

In order to capture hepatocellular carcinoma (HCC) cells in circulating peripheral blood, we made analysis to see if a -fetoprotein (AFP) mRNA exists in the peripheral blood obtained from patients with HCC and also, as a control, from hepatitis-viral-marker-positive patients without HCC and a healthy volunteer. As the number of HCC cells in peripheral blood and the quantity of AFP mRNA are expected to be very small, the analysis was performed by the reverse transcription followed by an original three-step polymerase chain reaction. By this highlysensitive method, 5 of 7 HCC patients were positive for AFP mRNA. These 5 positive patients consisted of three with clinically apparent recurrence, one preoperative patient with tumor thrombus in the portal vein and one recurrence-free patient who developed clinically detectable recurrence three months after this analysis. Neither 4 patients with positive viral markers nor a healthy volunteer was positive. The results suggest that detection of AFP mRNA from HCC patients' peripheral blood by our highlysensitive RT-PCR may be a practical and powerful tool to diagnose the preoperative spreading of HCC and to monitor its recurrence.
In conjunction with strategies introduced in recent years to identify cancer micrometastasis through amplification of cancer-associated mRNA, we developed a highly sensitive system to detect [alpha] -fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients. The aim of the present study was to make our original system quantitative. Peripheral venous blood from patients with hepatocellular carcinoma and [alpha] -fetoprotein-producing gastric carcinoma was subjected to reverse transcription followed by our original three-step polymerase chain reaction co-amplifying both the original sequence and our synthetic competitor. We succeeded in modifying our system for quantitative analysis, and investigated the perioperative change, the postoperative change and the change after chemotherapy in order to illustrate the possible application of this method. The quantitative analysis of [alpha]-fetoprotein mRNA present in the peripheral blood represents a useful tool for analyzing the relationship of surgery to recurrence, the effect of chemotherapy, and to predict impending recurrence in patients with hepatocellular and [alpha]-fetoprotein-producing gastric carcinomas.

For the detection of circulating colorectal carcinoma cells, we investigated the presence of cytokeratin 20 (CK 20) mRNA in the peripheral blood of colorectal carcinoma patients. Application of our published technique resulted in analysis by reverse transcription followed by three-step nested polymerase chain reaction. This analysis could detect a single Colo 205 colon cancer cell mixed with 1 ml of blood. Our system also successfully detected the presence of CK 20 mRNA in actual patients’ peripheral blood samples. Our highly sensitive and specific system for the detection of CK-20 mRNA from patients’ peripheral blood thus seems to be useful for screening for circulating colorectal carcinoma cells.

cDNA cloning from chick embryonic gonads subtracted from tissues of the brain, heart, liver, gizzard, mesonephros and skeletal muscle was performed to identify genes with expression unique to embryonic gonads. Several cDNA clones encoding characterized as well as many uncharacterized genes were obtained. ADP-ribosylation factor (ARF) of these identified genes was preferentially expressed in the chick embryonic ovary and testis as revealed by reverse transcription-polymerase chain reaction analysis. Expression of the ARF was evaluated through embryonic development, but no difference in the transcript (relative to glyceraldehyde-3-phosphate dehydrogenase transcript) was observed between the left and right ovaries, and
between the ovary and testis. In addition, the ARF transcript was detected in the gonads on embryonic days 5 to 21. These findings indicate that the ARF is constantly, but preferentially expressed in the embryonic gonads during development.


http://www.sciencedirect.com/science/article/B6T99-3X9YSFN-C/2/3370cafd4258c51d9079609e7f1413b8

We wanted to study the expression of P2-receptors at the mRNA-level in the heart and if it is affected by congestive heart failure (CHF). To quantify the P2 receptor mRNA-expression we used a competitive RT-PCR protocol which is based on an internal RNA standard. The P2 receptor mRNA-expression was quantified in hearts from CHF rats and compared to sham-operated rats. Furthermore, the presence of receptor mRNA was studied in the myocardium from patients with heart failure. In the sham operated rats the G-protein coupled P2Y-receptors were expressed at a higher level than the ligand gated ion-channel receptor (P2X1). Among the P2Y-receptors the P2Y6-receptor was most abundantly expressed (P2Y6 > P2Y1 > P2Y2 = P2Y4 > P2X1). A prominent change was seen for the P2X1- and P2Y2-receptor mRNA levels which were increased 2.7-fold and 4.7-fold respectively in the myocardium from the left ventricle of CHF-rats. In contrast, the P2Y1-, P2Y4- and P2Y6-receptor mRNA levels were not significantly altered in CHF rats. In human myocard the P2X1-, P2Y1-, P2Y2-, P2Y6- and P2Y11-receptors were detected by RT-PCR in both right and left atria and ventricles, while the P2Y4-receptor band was weak or absent. In conclusion, most of the studied P2-receptors were expressed in both rat and human hearts. Furthermore, the P2X1- and P2Y2-receptor mRNA were upregulated in CHF, suggesting a pathophysiological role for these receptors in the development of heart failure.


ATP causes the activation of p38 or ERK1/2, mitogen activated protein kinases (MAPKs) resulting in the release of tumor necrosis factor-[alpha] (TNF) and Interleukin-6 (IL-6) from microglia. We examined the effect of TNF and IL-6 on the protection from PC12 cell death by serum deprivation. When PC12 cells were incubated with serum-free medium for 32 hr, their viability decreased to 30 %. IL-6 alone slightly protected the death of PC12 cells, whereas TNF alone did not show any protective effect. In the meanwhile, when PC12 cells were pretreated with TNF for 6 hr and then incubated with IL-6 under the condition of serum-free, the viability of PC12 cells dramatically increased. TNF induced an increase of IL-6 receptor (IL-6R) expression in PC12 cells at 4-6 hr. These data suggested that 6 hr pretreatment with TNF increased IL-6R expression in PC12 cells, leading to an enhancement of IL-6-induced neuroprotective action. To elucidate the role of p38 in pathological pain, we investigated whether p38 is activated in the spinal cord of the neuropathic pain model. In the rats displaying a marked allodynia, the level of phospho-p38 was increased in the microglia of injury side in the dorsal horn. Intraspinal administration of p38 inhibitor suppressed the allodynia. These results demonstrate that neuropathic pain hypersensitivity depends upon the activation of p38 signaling pathway in microglia in the dorsal horn following peripheral nerve injury.

http://www.sciencedirect.com/science/article/B6T99-487F0PJ-2/2/4938700a27ee12f04f24566883c266c9

Uncoupling proteins (UCPs) are supposed to be involved in diet-induced thermogenesis. Their activities are usually elevated by feeding and reduced by fasting in normal animals. To investigate whether fasting affects the expression of UCPs mRNA in brown adipose tissue (BAT) of bilateral ventromedial hypothalamus (VMH)-lesioned rats, we determined the gene expression of UCP1, UCP2 or UCP3 in BAT of VMH-lesioned rats and examined oxygen consumption in these rats under fed or 48-h fasted conditions. Northern blotting revealed no difference in the expression of UCPs mRNA in BAT between VMH-lesioned and sham-operated rats under the fed condition, however, expressions were increased markedly in BAT of VMH-lesioned rats under the fasted condition. Under the fed condition, no difference in oxygen consumption was observed between VMH-lesioned and sham-operated rats. Under the fasted condition, oxygen consumption decreased in both rats, however, it decreased in VMH-lesioned less than in sham operated rats. To explore the mechanism that fasting elevated BAT UCPs mRNA in VMH-lesioned rats, we measured peroxisome proliferator-activated receptor (PPAR)-[gamma] mRNA and protein in BAT, because PPAR-[gamma] agonist can elevate UCPs mRNA levels in BAT. Under the fed condition, no differences in the expression of PPAR-[gamma] mRNA and protein content were observed between in BAT of VMH-lesioned and sham-operated rats. Under the fasted condition, however, both increased in BAT of VMH-lesioned rats. These results suggest that VMH-lesions enhance the gene expression of UCPs in BAT under long-term fasting as a defensive reaction to inhibit the reduction of body temperature through an increase in PPAR-[gamma] activity.


http://www.sciencedirect.com/science/article/B6T99-49STN7H-3/2/6e893ea6358ff4848b9dad50ce6aacb0

It has previously been shown that mice with a defect in Fas ligand-mediated apoptosis have an enhancement of ectopic bone formation. We investigated the expression of bone-related markers - alkaline phosphatase, collagen, bone sialoprotein, osteocalcin, osteopontin, and bone morphogenetic proteins (BMP) -2, -4, and -7; and cytokines interleukin-1[alpha] (IL-1), IL-1[beta], and tumor necrosis factor-[alpha] (TNF-[alpha]) in ectopic new bone induced by recombinant human (rh) BMP-2 in mice without functional Fas-ligand (gld mice). At day 6 after rhBMP-2 implantation, gld mice formed more cartilage and mesenchyme compared with their wild type littermates. At later stages, gld mice did not differ from the control mice in the volume of newly formed tissue, expressing higher level of BMP genes and lower levels of genes involved in osteoblast maturation - bone sialoprotein and osteopontin. Differences in the levels of expression of IL-1[alpha] and TNF-[alpha] were observed only at day 12 after rhBMP-2 implantation. These results suggest that gld mice have an increased recruitment of cells of mesenchymal origin and an abnormal pattern of differentiation and maturation of the newly formed mesenchymal tissues.


http://www.sciencedirect.com/science/article/B6T99-3RHNJK5-
The rat model of acute gastric damage induced by ischemia-reperfusion (I-R) has been used to evaluate the protective effect of various drugs on gastric injury. However, the quantitative expression state of cyclooxygenase-2 (COX-2), a protein which induces cytoprotective prostaglandins during inflammation, is still unknown in acute gastric injury induced by I-R. Therefore, we have quantitatively investigated the level of expression of COX-2 mRNA in injured gastric tissue of this model using the reverse transcription-competitive polymerase chain reaction method. The mRNA for COX-2 was expressed at low or undetectable levels in the normal gastric tissues in control rats, which were fasted for 18 hrs without I-R. The mRNA levels of COX-2 in injured gastric tissues were higher than those of control tissues between 6 hrs and 48 hrs after I-R. The maximum expression of COX-2 mRNA was recorded at 24 hrs (approximately a 200-fold increase). The expression state of COX-2, which has been ascertained in this study, should be useful in evaluating the effect of various drugs on the expression of COX-2 in acute gastric damage.


http://www.sciencedirect.com/science/article/B6T99-490H4NG-1/2/792c1e218beb6e76ecf29256ce844f35

The changes of methylation status of various gene promoters are a common feature of malignant cells and these changes can occur early in the progression process. Therefore, abnormal methylation can be used as cancer marker. Such studies will first require the development of a panel of methylated markers that are methylated in cancer tissues but unmethylated in normal tissues or methylated status is different between cancer tissues and normal tissues. By using methylation-specific PCR (MSP) assay method, we observed alterations in DNA methylation at the double promoter regions of the progesterone receptor (PR) gene and estrogen receptor (ER[alpha]) gene in various tumor cell lines. Compared with normal white blood cell, the methylation status of PRA promoter in various cancer cell lines changed from unmethylation pattern to methylation pattern. That of PRB promoter changed from both unmethylated and methylated alleles to only methylated allele. The methylation status of ER[alpha]-A and ER[alpha]-B promoter in various cancer cell lines are cell-specific. This study indicates that PR promoter methylation may be a molecular marker in various cancer detections. And the methylation status of ER[alpha]-A and ER[alpha]-B is cell-specific.


http://www.sciencedirect.com/science/article/B6T99-3YXB9SD-1/2/714141c13f5ebaf4156571a6d0ac2a99

Recent data has demonstrated that cacao liquor polyphenols (procyanidins) have antioxidant activity, inhibit mRNA expression of interleukin-2 and are potent inhibitors of acute inflammation. Given the widespread ingestion of cocoa in many cultures, we investigated whether cocoa, in its isolated procyanidin fractions (monomer through decamer), would modulate synthesis of the pro-inflammatory cytokine, interleukin-1[beta]. Both resting and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) were investigated at the levels of transcription and protein secretion. Individual cocoa fractions were shown to augment constitutive IL-1[beta] gene expression, although values varied between subjects. Interestingly, the smaller fractions of cocoa
(monomer-tetramer) consistently reduced IL-1[beta] expression of PHA-stimulated cells by 1-15%, while the larger oligomers (pentamer-decamer) increased expression by 4-52%. These data, observed at the transcription level, were reflected in protein levels in PHA-induced PBMC. The presence or absence of PHA did not alter the effects of the cocoa procyanidins with the exception of the pentamer. This study offers additional data for the consideration of the health-benefits of dietary polyphenols from a wide variety of foods, including those benefits associated specifically with cocoa and chocolate consumption.


http://www.sciencedirect.com/science/article/B6T99-3W2T831-6/2/2f8bf146ca53c502e0d6302462c6e5ac

In the present study we have investigate whether cytokines are constitutively and differently expressed in intestine during the differentiative processes that take place at weaning. We have analyzed the expression of IL-1[beta], IL-2, IL-4 and IFN[gamma] by polymerase chain reaction in Peyer's patches (PP) and in intestine deprived of PP (I-PP) of rats from 16 to 30 days of age. The results showed a constitutive and marked expression of the cytokines already before weaning, with the exception of IL-2 in PP and IFN[gamma] in I-PP. IL[-beta] was the only cytokine to show a different expression at various ages with an initial increase at 19 days and a further elevation at 21 days when intestinal epithelium passes through major differentiative stages, suggesting an involvement of this cytokine in intestinal development. We have also tested whether treatment of rats with the immunosuppressor cyclosporin A (CsA) could affect intestinal differentiation. The results showed that only some markers of differentiation were affected (proliferation of staminal crypt cells and length of crypts). This was probably due to a direct effect rather than an immunomeditated effect of CsA, since treatment of three intestinal cell lines (Caco-2, HT-29, FRIC) with CsA indicated that this drug can exert a cytostatic activity on intestinal cells.


http://www.sciencedirect.com/science/article/B6T99-49H6BGJ-3/2/731d1f2fc76fbed391d32945c0e9ae05

Since endotoxin lethality is enhanced by Mg deficiency in animals, we determined whether endotoxin-induced vascular hyporeactivity to phenylephrine (PE) is enhanced in Mg-deficient rats. Normal and Mg-deficient adult male Wistar rats were injected with Escherichia coli 011: B4 lipopolysaccharide (1 or 5 mg/kg, i.p.). Six h later, rings prepared from their thoracic aortas showed severe hyporeactivity to PE. This was more pronounced in the Mg-deficient rats, and was reversed by in vitro treatment with a highly selective inducible nitric oxide (NO) synthase inhibitor, 1400 W, or a highly selective soluble guanylyl cyclase inhibitor, ODQ. However, reversal required high doses of both inhibitors in Mg-deficient rats. Endotoxemia for 6 h was associated with elevated serum interleukin (IL)-1[beta] and tumor necrosis factor (TNF)-[alpha] levels, and strong TNF receptor mRNA expression in the abdominal aortas, which were significantly greater in the Mg-deficient rats. Treatment of the thoracic aortas, isolated from control and Mg-deficient rats before endotoxic challenge, with IL-1[beta] or TNF-[alpha] for 6 h in vitro caused hyporeactivity to PE, but its severity did not differ significantly between the two groups. These results suggest that high serum IL-1[beta] and TNF-[alpha] levels, and increased TNF receptor production in the vascular tissue, contribute to vascular hyporeactivity to PE in endotoxemia, and to its enhancement in Mg-deficient rats, via NO/cGMP signaling.

http://www.sciencedirect.com/science/article/B6T99-3X05HTX-11/2/e914656134de1d8140d842a2ccee532c8

Tumor necrosis factor-[alpha] (TNF-[alpha]) is an important mediator of insulin resistance in obese subjects, through its overexpression in fat tissue. However, how exercise can modify the expression of TNF-[alpha] is controversial. We examined TNF-[alpha] in adipose tissue using an animal model of insulin resistance that was produced by feeding rats a diet high in sucrose. The rats were allocated to one of three groups: those receiving a starch-based diet (control group); those fed a high-sucrose diet (sucrose-fed group); and those fed a high-sucrose diet and given wheel exercise (exercised group). The animals were allowed to eat and drink ad lib for 4 or 12 weeks (4 wk: control N = 7, sucrose-fed N = 7, exercised N = 10; 12 wk: control N = 5, sucrose-fed N = 5, exercised N = 9). The voluntary wheel exercise was initiated with the feeding of the high-sucrose diet. The rats in the exercise groups ran 15 +/- 3 km/week. We showed that 12-week voluntary running exercise significantly (P < 0.05) and free fatty acid (0.98 +/- 0.07 mEq/L vs 1.4 +/- 0.05 mEq/L) concentrating in portal vein blood were reduced compared to sucrose-fed group. The amounts of fatty tissue both in mesenteric and subcutaneous tissues were significantly (P < 0.05) decreased through running exercise. We consider that up-regulation of TNF-[alpha] in mesenteric fat may be a compensatory mechanism for the reduction of fatty acid in adipose tissues and this change could control metabolic homeostasis during exercise to modulate a hyperinsulinemic state.


http://www.sciencedirect.com/science/article/B6T99-3YYTH6C-3F/2/edc23366ae0c793fbaa6989260c5f20c

To elucidate whether GHR and GHBP are coordinately regulated or not, we studied the effect of fasting with or without GH administration on the GHR and GHBP mRNAs in the liver as well as in extrahepatic tissues in rats. Tissues were collected from 7-week-old male rats by decapitation 1, 3, and 7 days after the start of fasting. Liver GHR mRNA levels were not affected 1 day after the start of fasting but progressively decreased for the subsequent 3 and 7 days of fasting as compared with those in control rats fed ad libitum. In contrast, liver GHBP mRNA levels significantly rose after 1 day fasting, returned to the control level after 3 days and further reduced after 7 days of fasting. Changes in GHBP mRNA level after fasting were different among the tissues. A transient increase in GHBP mRNA levels was observed in muscle and heart as well as liver, while the GHBP mRNA levels in fat tissues did not change throughout 7 days of fasting. Next, bovine GH(bGH) was administered ip to the fasted rats and control fed rats for either 1 day(100mg, tid) or 5 days(150mg, daily). In fed rats, liver GHR mRNA level was significantly increased by 1 day bGH treatment, but after 5 days treatment with bGH it was not different from the level in saline-injected control. Accordingly, net increment of plasma IGF-I was 296.0 ng/ml with 1 day bGH treatment and 234.2 rig/ml with bGH administration for 5 days. In fasted rats, liver GHR mRNA level did not changed after 1 day treatment with bGH, but markedly decreased 5 days after bGH administration. Net increment of plasma IGF-I was slightly reduced to 284 ng/ml with 1 day treatment with bGH, and markedly decreased to 37.0 with bGH administration for 5 days. The effect of GH administration on liver GHBP mRNA level was virtually absent in either fasting or fed state. These findings suggest that GHR and GHBP mRNAs in the liver are expressed in different ways and that expression of GHBP mRNA is differently regulated among
Alternative splicing patterns of cyclic AMP response element-binding protein (CREB) in dorsal root ganglia, lumbar sympathetic ganglia and several peripheral tissues of the rat have been investigated by an exon-flanking polymerase chain reaction strategy. A series of RT-PCR with primer pairs flanking all possible alternative splicing sites (corresponding to a genomic region with at least one full exon and two flanking introns) has revealed multiple tissue specific splice variants. These include some novel transcripts that lack the phosphorylation site and part of the leucine zipper region which is crucial for dimerization and DNA binding. Some isoforms previously reported as testis-specific were also detected in rat peripheral ganglia and other tissues. Notably, splicing patterns are specific for some regions. Some of the splice variants indicate inhibitory functions due to lacking phosphorylation sites or partially missing DNA-binding or leucine zipper domains. These findings suggest a complex expression and functional regulation of CREB in peripheral tissues including dorsal root and sympathetic ganglia.

We examined the effects of diabetes on the morphological features and regenerative capabilities of adult mouse nodose ganglia (NG) and dorsal root ganglia (DRG). By light and electron microscopy, no apoptotic cell death was detected in the ganglia obtained from either streptozotocin (STZ)-induced diabetic or normal C57BL/6J mice in vivo. Neurite regeneration from transected nerve terminals of NG and DRG explants in culture at normal (10 mM) and high (30 mM) glucose concentrations was significantly enhanced in the diabetic mice. Chromatolytic changes (i.e. swelling and migration of the nucleus to an eccentric position in the neurons, and a loss of Nissl substance in the neuronal perikarya) and apoptotic cell death (less than one-fifth of the neurons) in the cultured ganglia were present, but neither hyperglycemia in vivo nor high glucose conditions in vitro altered the morphological features of the ganglia or the ratios of apoptotic cells at 3 days in culture. By semiquantitative RT-PCR analysis, the mRNA expressions of ciliary neurotrophic factor (CNTF) in DRG from both mice were down-regulated at 1 day in culture. The expression in diabetic DRG, but not in control DRG, was significantly up-regulated at later stages (3 and 7 days) in culture. In summary, hyperglycemia is unlikely to induce cell death in the sensory ganglia, but enhances the regenerative capability of vagal and spinal sensory nerves in vitro. The up-regulation of CNTF mRNA expression during the culture of diabetic DRG may play a role in the enhanced neurite regeneration.

The mutations of the SCN5A gene have been implicated to play a pathogenetic role in Brugada syndrome, which causes ventricular fibrillation. To determine the Brugada-associated mutations in Japanese patients, facilitate pre-symptomatic diagnosis, and allow genotype-phenotype studies, we screened unrelated patients with Brugada syndrome for mutations. DNAs from 6 Japanese patients were obtained and the sequence in the translated region of SCN5A was determined. We could not find the mutations reported previously, but found 17 sites of nucleotide change, consisting of 7 synonymous and 10 non-synonymous nucleotide changes in our patients. Among them, two non-synonymous nucleotide changes (G1663A and G5227A) are specific to our patients and these changes were not found in 53 healthy controls. In 4 patients out of 6, no specific nucleotide change for Brugada syndrome could be detected. Our findings demonstrating no patient-specific change in the translated region of the SCN5A gene among two thirds of the small number of patients examined here imply that another gene other than the SCN5A may be associated with this disease, supporting previous investigations in Japan and other countries.


To explore a role of the transiently appearing cellular retinol-binding protein, type II (CRBP(II)) in perinatal chick liver, we have examined whether the relationships exist among the perinatal changes in hepatic CRBP(II) protein and mRNA levels, retinal reductase activity and [beta]-carotene levels in liver and serum. Northern blot analysis for hepatic CRBP(II) revealed a transient expression of CRBP(II) mRNA around hatching. The protein of CRBP(II) was also expressed transiently and the highest levels of CRBP(II) were found in the livers 1-3 days after birth. The retinal reductase activity was very low at embryonic age, but its activity rapidly rose at hatching, peaking at 1 day after birth, followed by a gradual decrease to a lower level in 7-day-old chicks. This perinatal pattern of the retinal reductase activities was similar to the pattern of transient appearance of the hepatic CRBP(II), and was also paralleled to the developmental changes in serum and liver [beta]-carotene concentrations. These findings suggest that hepatic CRBP(II) transiently appearing during the perinatal period may involve in metabolizing hepatic [beta]-carotene, directing the retinal to the retinal reductase and leading further to the subsequent esterification of the converted retinol.


Calcitonin gene-related peptide (CGRP) is one of the major neuropeptides released from sensory nerve endings and neuroendocrine cells of the lung. Two CGRP isoforms, [alpha]- and [beta]-CGRP, have been identified in rats and humans, but no studies have attempted to reveal direct evidence of differences in action or location of these isoforms in allergic inflammation (AI). We investigated mRNA expressions of [alpha]- and [beta]-CGRP in lungs, nodose ganglia (NG), and dorsal root ganglia (DRG) of an animal model for AI of the airways, utilizing a model created by sensitizing Brown Norway (BN) rats with ovalbumin (OVA). By semiquantitative RT-PCR analysis,
long-lasting enhanced expression of the [beta]-CGRP mRNA was shown in the lungs of the AI rats (14.5-fold enhancement at 6 hr, 8.1-fold at 24 hr, and 3.7-fold at 120 hr after OVA-challenge compared to the level in the lungs of phosphate-buffered saline (PBS)-challenged control rats). In contrast, the mRNA expression of the [alpha]-CGRP in AI lungs showed only a transient increase after OVA-challenge (2.7-fold at 6 hr) followed by a lower level of expression (0.5-fold at 48 hr and 0.6-fold at 120 hr). The mRNA expressions of both isoforms in NG, but not in DRG, were transiently up-regulated at 6 hr after antigen challenge. In situ RT-PCR in combination with immunohistochemical analysis revealed that [beta]-CGRP was expressed in neuroendocrine cells in clusters (termed neuroepithelial bodies [NEBs]) in AI lungs. These results indicate that the long-term induction of [beta]-CGRP in NEBs may play an important role in pulmonary AI such as bronchial asthma.


http://www.sciencedirect.com/science/article/B6T99-47548Y8-J9/2/dc9a0f329fed636149e1588fa867f2a6

A full-length cDNA clone for rabbit tryptophan hydroxylase (TPH) was modified and subcloned into a bacterial expression vector. Expression of this gene in the protease-deficient strain of bacteria, BL21[DE3], produced TPH immunoreactive protein which exhibited enzyme activity. Treatment of the recombinant enzyme (in bacterial extracts) with the purified catalytic subunit of the cAMP-dependent protein kinase and [y-32P]-ATP resulted in specific phosphorylation of TPH. This expression system provides a means of generating and purifying large amounts of this important enzyme. Moreover, these experiments establish that TPH will serve as an in vitro substrate for cAMP-dependent protein kinase.


The expression of ORL1 receptor mRNA splice variants is determined in peripheral sensory and sympathetic ganglia and compared to mRNA expression for the three classic opioid receptor subtypes (mu, delta, and kappa) using the method of reverse transcription-polymerase chain reaction. ORL1, mu, delta and kappa receptor subtype mRNAs are present in human dorsal root ganglia (DRG) and trigeminal ganglia and rat DRG. ORL1, mu and delta receptor subtype mRNAs are present in rat superior cervical ganglia and only ORL1 and delta receptor mRNAs are present in rat lumbar sympathetic ganglia. Both the ORL1 mRNA splice variants are present in sensory and sympathetic ganglia, however, expression of the shorter ORL1 receptor mRNA dominates over expression of the longer splice variant in rat brain and DRG, whereas, expression of the longer splice variant is dominant in sympathetic ganglia.


http://www.sciencedirect.com/science/article/B6T99-474YKY4-1DP/2/2be6dbe850711eed068bb3dbae6a7f12
Gastrin is mitogenic for several colon cancers and is postulated as an autocrine growth factor for colon cancer cells. In the present study we report the development of a simple competitive polymerase chain reaction (PCR) method for measuring relative abundance of gastrin gene expression in colon cancer cells. Primers flanking exons 2 and 3 of the gastrin gene were utilized for co-amplification of cDNA and genomic DNA. The amplification of genomic DNA was distinguished from that of cDNA by the presence of the 130 bp intron sequence which was resolved by electrophoresis on agarose gels. A standard reaction of competitive PCR, using known concentrations of genomic DNA and cDNA, was first established. The steady state levels of gastrin mRNA were next quantitated in three human colon cancer cell lines (HCT-116, Colo-205 and DLD-1) by competitive PCR. Gastrin mRNA levels in these cell lines ranged from ~0.1 to 1.0 fmoles/mg total RNA (~2-25 copies of gastrin mRNA per cell). This low to moderate levels of gastrin were expressed by human colon cancer cell lines which may function as autocrine growth factors for colon cancers.


To investigate the expression of aryl hydrocarbon receptor repressor (AhRR) and related molecules in various tissues and the effects of aromatic hydrocarbons (AHs) on their expression, we developed a reliable technique of quantification of human AhRR as well as aryl hydrocarbon receptor (AhR), AhR nuclear translocator (ARNT) and cytochrome P450 1A1 (CYP1A1) mRNA by real-time TaqMan PCR method. First, we examined the expression of these genes in human adult or fetal tissues. The levels of AhRR expression were extremely high in testis, very high in lung, ovary, spleen and pancreas from adults, whereas those were low in those from fetuses. On the other hand, CYP1A1 expression was extremely high in lung, and AhR and ARNT were ubiquitously expressed in almost all tissues. Second, we compared the expression levels of these genes in mononuclear cells (MNCs) from various sources. Comparison of the basal expression levels of these genes in MNCs demonstrated that MNCs from umbilical cord blood showed higher AhRR or CYP1A1 expression than those from adults. The induction of AhRR or CYP1A1 expression by 3-methylcholanthrene (3-MC) was observed in MNCs from adults but not from umbilical cord blood. Consequently, there existed characteristic differences in the basal levels of AhRR and CYP1A1 expression in MNCs, as well as in their inducibility by 3-MC among MNCs from various types of human bloods. These results will provide basic information for a possible application of AhRR and CYP1A1 measurements to evaluate AH exposure in vivo.


To determine whether rat retinal photoreceptor cells produce inhibin, a molecule closely related to activin, a multifunctional growth factor in the transforming growth factor [beta] superfamily (TGF[beta]), we have conducted immunohistochemistry using specific antibodies for inhibin which were raised against a synthetic N-terminal fragment of the [alpha]-subunit of inhibin. The mature inhibin molecule was identified at both the inner and outer segments of photoreceptor cells. To determine if messenger RNA for the [alpha]-subunit of inhibin is expressed in the retinal cells, both in situ hybridization with a specific probe and the reverse transcription-polymerase chain
reaction (RT-PCR) technique with primers specific for the [alpha]-subunit of inhibin were used. Messenger RNA expression of the [alpha]-subunit of inhibin was detected by RT-PCR and localized in the photoreceptor cells as determined by in situ hybridization. In addition, the identity of the cDNA product of RT-PCR was verified with Southern analysis and DNA sequencing. The localization of mature inhibin protein and its corresponding message to photoreceptor cells suggest that inhibin may have a paracrine function in the retina, perhaps in the photoreceptor cells themselves.

Livestock Production Science (2)


The global biodiversity crisis extends to autochthonous local breeds of livestock. There is an increasing danger that these rare breeds become extinct and with them their locally adapted gene pool. Modern molecular tools such as parentage testing using microsatellite genotyping are powerful in guiding management and conservation. We tested nine microsatellite markers in three Croatian horse breeds and obtained high exclusion probabilities (EPs) for the most common test scenario 'one parent and offspring known and the other parent tested' (99.9% in Posavina and Croatian Coldblood and 99.3% in Lipizzaner), despite that Lipizzaner has an overall lower genetic variability at microsatellite loci. To become a useful tool in breed management in countries with developing economies, genetic screening systems must be designed to be statistically powerful yet economically viable. Therefore, a suite of six markers that can be run in two multiplex systems and which still gives high exclusion probabilities (99.5% in Posavina and Croatian Coldblood and 98% in Lipizzaner) was chosen.


http://www.sciencedirect.com/science/article/B6T9B-44MFPWG-3/2/52f2e816cde7df989cc9534e7d71b6e

We investigated a relation between carcass lipid contents and plasma leptin in 40 Japanese Black steers, which were fed a 12% crude protein diet (CP12) or a 16% crude protein diet (CP16) in the growing phase (from 10 to 18 months of age) and a low starch level diet (LS) or a high starch level diet (HS) in the finishing phase (from 19 to 27 months of age). Plasma leptin concentrations were measured by multi-species leptin radioimmunoassay kit using recombinant bovine leptin as the standard. The CP16 group had greater backfat thickness at slaughter compared with the CP12 group (PPPPr=0.59, P<0.05). However, plasma leptin levels seem to be weak index to predict final adiposity in Japanese Black steers.

Summary
It is generally assumed that squamous cell carcinoma develops in a stepwise manner from normal bronchial epithelium towards cancer by the accumulation of (epi)genetic alterations. Several mechanisms including mutations and homozygous deletions or hypermethylation of the p16INK4a promoter region can cause loss of p16 expression. Recent studies suggest overexpression of the polycomb-group gene BMI-1 might also down-regulate p16 expression. In this study, we analyzed the p16 expression in relation to the methylation status of the p16 promoter region of the p16INK4a gene and the expression of BMI-1 in bronchial squamous cell carcinomas (SCC) and its premalignant lesions. Nine (69%) SCC showed loss of p16 expression and 10 (77%) showed expression of BMI-1. Of four p16 positive samples two (50%) were BMI-1 positive, whereas among nine p16 negative samples, eight (89%) revealed BMI-1 staining. Four (44%) p16 negative samples were hypermethylated at the p16INK4a promoter region; the other p16 negative tumors that showed no hypermethylation revealed BMI-1 staining. Only two premalignant lesions showed absence of p16 expression, of which one (carcinoma in situ) was hypermethylated at the p16INK4a promoter region and the other (severe dysplasia) showed BMI-1 expression. In total, 11 precursor lesions (48%) revealed BMI-1 expression. In conclusion, the results of this study suggest that loss of p16 expression by promoter hypermethylation is inconsistently and occurs late in the carcinogenic process at the level of severe dysplasia. To what extent overexpression of the polycomb-group protein BMI-1 attributes to down regulating of p16 expression remains unclear.


http://www.sciencedirect.com/science/article/B6T9C-480CRM1-2/2/cf958b9032120f2627c7f5f28fb15014

The p53 gene is frequently mutated in lung tumors, and mutations may be caused by both polycyclic aromatic hydrocarbons (PAHs) and nitrosamines found in tobacco smoke. The two major forms of lung cancer, adenocarcinoma (AC) and squamous cell carcinoma (SCC), are known to differ in the proportion of tumors exhibiting p53 mutation, and may also differ in the mutational spectra produced. Previous studies comparing p53 mutational spectra between AC and SCC of the lung have been limited by small sample size. We examined p53 mutations in exons 5-8 in 202 cases of AC and 82 cases of SCC from smoking lung cancer patients in the Western Pennsylvania region. The percent of cases with p53 mutation was significantly lower in ACs (40/202, 20%) compared to SCCs (29/82, 35%, P=0.006). The proportion of mutations present that were G to T transversions was not significantly different between the two tumor types (52% of p53 mutations in AC compared to 32% in SCC). G to A transitions either did not differ in frequency in the two types of lung cancer (20% of mutations in AC and 24% of mutations in SCC). A distinct spectrum was observed, however, in the p53 mutation pattern in the two types of lung cancer. ACs showed a strong preference for a mutational hotspot at codons 248 and 249, while squamous cell tumors showed mutational events spread throughout exons 5-8, with a preference for codon 267. Mutations at codon 267 in SCC were all C to T transitions that occurred at CpG sites. Both tumor types demonstrated preferential mutation of the non-
transcribed strand (100% of all G to T transversions and 55% of the G to A transitions). These results suggest that p53 mutations in both types of lung tumors may arise from adduction by both PAHs and nitrosamines. Mutations arising in ACs appear selectively in regions of p53 that produce more rigid proteins, suggesting a drastic change in p53 function is needed to result in ACs, while less constrained changes in p53 function can result in SCCs. Mutation in p53 was not found to be related to patient survival in this group of patients, while tumor size and degree of differentiation were poor survival predictors.


http://www.sciencedirect.com/science/article/B6T9C-4B4VPNH-2/2/92db0969cc1b9672e3d02e5b779c1463

Early diagnosis of lung carcinoma is greatly desired. A potential source of early information regarding the process of cancerisation in the airways is examining DNA from the area of chronic damage, i.e. airways and lung parenchyma. We therefore investigated DNA in EBC of patients with NSCLC and healthy volunteers. Human DNA was amplified by PCR in exhaled breath condensate and used to detect p53 mutations. A PCR of the [beta]-actin gene fragment was used to detect human DNA in each of the EBC samples. In 65.7% of the samples, the [beta]-actin gene was found. Extracted DNA as well as native EBC were equally suited as starting material for amplification. Mutations of the p53 gene were investigated in all EBC samples of NSCLC patients. p53 exons 5-8 were amplified using nested PCR and subsequently sequenced. Mutations were found in four of the patients (n=11; 36.4%) while no mutation was found in volunteers (n=10). Mutations detected in EBC were also compared with those of corresponding tumor tissue. Different point mutations in EBC and tumor tissue were revealed in all cases. Our findings demonstrate that exhaled breath condensate may be used for analysis of somatic gene mutations in an area of direct tobacco-related DNA damage.


http://www.sciencedirect.com/science/article/B6T9C-3VCKRM0-6/2/eeaa0a27e5a02d1bf2f81b7179d520e

Melanoma tumor antigens, MAGE-1 and -3 are presented on HLA-A1 and -Cw*1601, or -A1 and -A2, respectively, to the corresponding cytotoxic T lymphocytes (CTL). If CTL recognizing these antigens were generated in patients, clones of positive tumor cells should be eliminated. To ascertain whether such an immunological response is active in patients with lung cancer and to determine what fraction of lung cancer patients are candidates for MAGE oriented immunotherapy, we assessed the relationship between HLA-A1 or -A2 expression and MAGE-1 or -3 gene expression in their tumors. MAGE-1 and -3 were detected in 18/55 (33%) and 23/55 (42%), respectively, by reverse transcriptase (RT)-polymerase chain reaction (PCR). Allele specific PCR revealed HLA-A1 and -A2 alleles to be expressed in 0/55 (0%) and 22/55 (40%) of our cohort, respectively. Among the 22 patients with HLA-A2 genotype, expression of HLA class I antigens detectable by immunohistochemistry was lost in five (23%) cases. The frequency of MAGE-3 expression in HLA-A2 patients was 5/17 (29%), somewhat lower than that of patients without HLA-A2 expression, 18/38 (47%), although the difference was not statistically significant (P=0.17). Neither was there a significant association between HLA-A2/MAGE-3 co-expression and survival (P=0.15, logrank test). We conclude that there is no clear evidence for elimination of lung cancers co-expressing HLA-A2 and MAGE-3 in vivo. Approximately 10% (5/55) of Japanese
l lung cancer patients are potential candidates for MAGE-3-based immunotherapy.

http://www.sciencedirect.com/science/article/B6T9C-4F6MDHH-2/2/be2ce66bcd2f12a7a0217340b75d56b

Summary
YKL-40 is a 40 kDa protein with possible involvement in tissue remodeling, cell proliferation and angiogenesis. Elevated serum YKL-40 levels in patients with metastatic cancers (including small cell lung cancer (SCLC)) are associated with poor prognosis. The aim of this study was to identify the cellular source of YKL-40 in SCLC patient biopsies and in a panel of 20 human SCLC lines cultured in vitro and in vivo in nude mice. In general, the SCLC cell lines had no or very limited (human) YKL-40 expression, whereas, by RT-PCR a pronounced murine (i.e., stromal) YKL-40 expression was present in all tumors. YKL-40 mRNA transcripts were detected by in situ hybridization in 9 of 10 biopsies from SCLC patients, and in each case the signal was localized in the peritumoral stroma in cells of typical macrophage morphology (confirmed by a CD68 macrophage specific stain). No YKL-40 mRNA expression was found in the cancer cells, in macrophages infiltrating the solid tumor areas, or in non-malignant tissue. In conclusion, the predominant source of elevated serum YKL-40 in SCLC is peritumoral macrophages.

http://www.sciencedirect.com/science/article/B6T9C-453598H-6/2/281ca568028ee048134c6febc3c89922

We established several in vitro drug-resistant cell lines after continuous, long-term exposure of each drug to elucidate mechanisms of drug resistance. Whether drug resistance in these in vitro resistant cell lines reflects clinical drug resistance still remains unanswered. In this study, a pair of lung cancer cell lines was established from one patient with squamous cell carcinoma of the lung, with one line being established before and one line after combination chemotherapy (cisplatin/ifosfamide/vindesine). Combination chemotherapy selected resistant EBC-2/R cells, which showed cross-resistance to 4-hydroxyifosfamide (3.2-fold), cisplatin (2.3-fold), and methotrexate (3.7-fold) and collateral sensitivity to vindesine (0.77-fold) compared with parent EBC-2 cells. EBC-2/R cells showed decrease in intracellular accumulation of cisplatin, increase in intracellular concentration of glutathione (GSH), and overexpression of multidrug resistance-associated protein (MRP) 3 when compared with EBC-2 cells. A single cycle of chemotherapy was not sufficient to select other mechanisms of drug resistance, such as multidrug resistance-1/P-glycoprotein, MRPs 1, 2, 4, and 5, lung resistance-related protein, metallothionein Il, glutathione S-transferase [p], [gamma]-glutamylcysteine synthetase (light and heavy chain), and excision repair cross complementing 1. Sequentially we established two cell lines, which cell lines showed the differences of the cisplatin resistance, expression level of MRP3, intracellular GSH level and intracellular accumulation of cisplatin. A pair of cell lines will be useful to elucidate resistant mechanisms of cisplatin in heterogeneous lung cancer cells.

http://www.sciencedirect.com/science/article/B6T9C-3W19GP9-
Programmed cell death (PCD) is a genetically regulated pathway that is altered in many cancers. This process is, in part, regulated by the ratio of PCD inducers (Bax) or inhibitors (Bcl-2). An abnormally high ratio of Bcl-2 to Bax prevents PCD, thus contributing to resistance to chemotherapeutic agents, many of which are capable of inducing PCD. Non-small cell lung cancer (NSCLC) cells demonstrate resistance to these PCD-inducing agents. If Bcl-2 prevents NSCLC cells from entering the PCD pathway, then reducing the amount of endogenous Bcl-2 product may allow these cells to spontaneously enter the PCD pathway. Our purpose was to determine the effects of bcl-2 antisense treatment on the levels of programmed cell death in NSCLC cells. First, we determined whether bcl-2 and bax mRNA were expressed in three morphologically distinct NSCLC cell lines: NCI-H226 (squamous), NCI-H358 (adenocarcinoma), and NCI-H596 (adenosquamous). Cells were then exposed to synthetic antisense bcl-2 oligonucleotide treatment, after which programmed cell death was determined, as evidenced by DNA fragmentation. Bcl-2 protein expression was detected immunohistochemically. All three NSCLC cell lines expressed both bcl-2 and bax mRNA and had functional PCD pathways. Synthetic antisense bcl-2 oligonucleotide treatment resulted in decreased Bcl-2 levels, reduced cell proliferation, decreased cell viability, and increased levels of spontaneous PCD. This represents the first evidence that decreasing Bcl-2 in three morphologically distinct NSCLC cell lines allows the cells to spontaneously enter a PCD pathway. It also indicates the potential therapeutic use of antisense bcl-2 in the treatment of NSCLC.


http://www.sciencedirect.com/science/article/B6T9C-4DYW52N-1/2/56eba500fd3aabbf03160f605bf42fb84

SummaryNeoangiogenesis is required for the growth of invasive lung carcinoma, however, the role of angiogenesis in the progression of premalignant changes to carcinoma of the lung is less clear. We have evaluated vascular endothelial growth factor (VEGF) expression and microvessel densities (MVDs) in 62 bronchoscopic biopsies from normal, reactive (basal cell hyperplasia (BCH)) and dysplastic bronchial epithelium and in tissue from twenty-seven invasive lung carcinomas in an effort to demonstrate angiogenic activity in these preneoplastic lesions and determine whether it is associated with increased bronchial epithelial VEGF expression. MVDs and VEGF RNA expression measured by quantitative RT-PCR were found to be elevated in comparison to normal bronchial tissue in bronchial dysplasias and invasive lung carcinomas but not in basal cell hyperplasias. Immunohistochemical (IHC) analyses revealed that expression of VEGF arose predominantly from bronchial epithelium. ELISA analysis of lung tumor tissue showed that elevated VEGF protein expression correlated with VEGF RNA levels ($r = 0.59$, $p = 0.004$). Increased expression of VEGF RNA was also found in histologically normal bronchial mucosa from patients with either dysplasia at other sites or a history of heavy tobacco use suggesting a possible field effect in regard to the elaboration of VEGF. Furthermore, analysis of VEGF isoforms and VEGF receptors by semi-quantitative RT-PCR in dysplastic and invasive lesions revealed characteristic altered patterns of expression in dysplasia and early cancer as compared to normal tissue. These results indicate that angiogenesis develops early in lung carcinogenesis and is associated with overexpression of VEGF.

LUN is a novel RING finger protein that is highly expressed in the lung and might be a transcriptional regulator of E-cadherin [J. Biol. Chem. 276 (2001) 14004]. It might be possible that LUN plays important roles in the development and progression of lung cancer through regulating expression of E-cadherin, but no clinical study on LUN expression has been reported. In the present study, we quantitatively examined gene expression of the LUN in surgical specimens resected from non-small cell lung cancer (NSCLC) patients. In normal lung tissues, the LUN gene expression was down-regulated in smokers (the mean LUN/GAPDH ratios, 0.222 for non-smokers and 0.144 for smokers; P=0.030). In addition, the mean LUN/GAPDH ratio in lung cancer tissues was significantly lower than that in normal lung tissues (0.072 versus 0.162; PLUN gene expression was slightly down-regulated along with progression of primary tumors, and strongly down-regulated along with nodal metastases (the mean LUN/GAPDH ratios, 0.091 for pN0, 0.073 for pN1, and 0.034 for pN2 diseases; P=0.001). These results suggested that LUN might play important roles in inhibition of nodal metastases as well as in suppression of smoking-related oncogenesis in NSCLC.


Mitotic checkpoint impairment is present in human lung cancers with chromosomal instability (CIN). Spindle-checkpoint genes have been reported to be mutated in several human cancers, but these mutations are infrequent. Recent reports suggest that the hBUBR1 gene may play an important role in mitotic checkpoint control and in mitotic checkpoint impairment in human cancers. We analyzed the expression of hBUBR1 in lung cancer cell lines using real time quantitative RT-PCR. The expression of BUBR1 was found to be up-regulated in all of these cell lines. In addition, we cloned and characterized the promoter region of hBUBR1 and determined its genomic structure, which includes 23 exons. The open reading frame (ORF) of the hBUBR1 gene comprises exons 1 through 23. There are GC-rich regions located at the flanking region and about 150 bp upstream from exon 1. The promoter region (424 bp upstream from exon 1) showed promoter activity and includes multiple transcription factor consensus binding motifs, including those for Sp1, Nkx-2, CdxA, SRY, MyoD, Iκ-2, HNF-3b, Staf, Oct-1, Nkx-2, v-Myb, and AML 1a. Multiple pathways leading to activation of those binding factors may contribute to hBUBR1 gene transcription. Knowledge of the genomic structure and the promoter region of the hBUBR1 gene will facilitate investigation of its role in mitotic checkpoint control and tumor progression in human cancers.


Cancer/testis (CT) antigens are considered promising candidates for vaccine-based immunotherapy. The aim of this study was to investigate which CT antigens should be targeted in immunotherapy of Japanese lung cancer. To determine the expression of 12 CT antigens in Japanese primary lung cancers and cell lines, a reverse-transcription polymerase chain reaction
(RT-PCR) analysis was performed. Among 46 primary lung cancers, high expression rates were found for MAGE-3 (41%, 19/46), and SSX-4 (35%, 16/46). A similar pattern of CT antigen expression was observed in 29 lung cancer cell lines. The expression frequency of a certain CT antigen, namely NY-ESO-1, in Japanese cases was drastically different from that in Caucasians. Polyvalent CT antigen vaccine may be effective to increase the number of lung cancer patients eligible for cancer-specific immunotherapy. Vaccination with MAGE-3 and SSX-1 would cover 57% of all patients, with three antigens, MAGE-3, SSX-1, and MAGE-4, would cover 65%, and with four antigens, MAGE-3, SSX-1, MAGE-4 and SSX-4, would cover 70%. Simultaneous expression of two or more CT antigens was observed in 25/46 (54%) primary lung cancers and 18/29 (62%) lung cancer cell lines. Polyvalent CT antigen vaccines may be also effective to reduce a chance of emergence of antigen loss variants, thus preventing tumors from escaping from the immune system. For this purpose, vaccination with combinations of MAGE-3 with MAGE-6, SSX-4, MAGE-1 or BAGE may be effective for a quarter of Japanese lung cancer patients. In addition, in silico surveys of dbEST database were used for identification of new CT antigens. We identified a novel gene, TES101RP, expressed only in some small cell lung cancers (SCLC) and in testis, as confirmed by RT-PCR analysis.


http://www.sciencedirect.com/science/article/B6T9C-48V964D-1/2/9a761b0002ab1d455068a5dd9e7b28e

Cigarette smoking is the dominant risk factor for lung cancer, but only a minority of smokers ever develops tumors. Though genetic susceptibility is likely to explain some of the variability in risk, results from previous studies of genetic polymorphisms have been inconclusive. As diet may also affect the risk of lung cancer, it is possible that the degree of risk produced by smoking and genetic susceptibility varies, depending on diet. To assess this hypothesis, we conducted a case-control study to examine the effect of cigarette smoking, dietary patterns and variation in genes involved in phase II metabolism. A total of 254 individuals with lung cancer and 184 healthy controls were recruited for the study. To identify persons with similar dietary patterns, cluster analysis was performed using nutrient densities of four major dietary constituents: protein, carbohydrate, animal fat, and dietary fiber. Two groups of individuals were identified with distinct dietary patterns: (1) a group (n=2241) with a high intake of animal fat and protein and a low intake of carbohydrates and dietary fiber (the 'healthy' pattern) (2) a group (n=197) with a high intake of fiber and carbohydrate and a low intake of protein and animal fat (the 'unhealthy' pattern). On stratified analysis, several genotype/dietary pattern combinations were found to affect risk of lung cancer. Smokers who were not homozygous for the most common GSTP1 allele and had a healthy dietary pattern were at significantly lower risk than smokers who were homozygous for the GSTP1 common allele and who had an unhealthy dietary pattern (OR=0.16, 95%CI: 0.04-0.57). Among smokers who were GSTM1 null, persons with a healthy dietary pattern were at lower risk than persons with an unhealthy dietary pattern (OR: 0.46, 95%CI: 0.21-1.01). Among smokers with an unhealthy dietary patterns, persons with a His/His genotype in the exon 3 polymorphism of EPHX1 were at significantly lower risk that persons who were not homozygous. These data suggest that dietary factors may affect the risk imposed by genetic susceptibility at detoxification loci. Adjustments using dietary pattern may be useful in elucidating the effects of polymorphisms in genes responsible for carcinogen metabolism.

The murine anti-bombesin monoclonal antibody, 2A11, has been demonstrated to inhibit growth of some small-cell lung cancer (SCLC) cells in nude mice xenografts and in a clinical trial. To determine if the expression of bombesin-like peptides (BLP) and their receptors (GRP-R and NMB-R) correlate with an in vitro response to 2A11, we measured these parameters in seven SCLC cell lines. Gastrin releasing peptide (GRP) mRNA was detected in three of seven cell lines (NCI-H69, NCI-H345, NCI-H510) and neuromedin B (NMB) mRNA was detected in all seven lines using an RNase protection assay (RPA). Immunoreactive BLP was detected in the cell pellets of all lines (range 0.11-59.90 pmol/mg protein) by a solid phase GRP radioimmunoassay (RIA) using 125I-labeled 2A11. RPA detected GRP-receptor mRNA in two cell lines (NCI-H69 and NCI-H345) and NMB-receptor in three lines (NCI-H345, NCI-H510, and NCI-H660). Reverse transcriptase-PCR confirmed the presence of receptor mRNA in these lines and detected NMB-receptor in an additional three lines (NCI-H69, NCI-H82, and NCI-H187). Calcium mobilization in response to BLP stimulation was detected in the six cell lines expressing either GRP-R or NMB-R mRNA but not in NCI-N417, which had no detectable BLP-receptor. 2A11 (5 µg/ml) inhibited colony formation by 26-61% after 2 weeks in all cell lines except NCI-N417. Thus, growth inhibition by 2A11 requires the presence of at least one BLP-receptor. These findings may be useful in selecting patients with SCLC for treatment with 2A11.


In order to clarify the anti-tumor activity of IFN-[gamma], we investigated the direct IFNfluence of IFN-[gamma] on both the growth and cell-surface antigen expression of tumor cells. In the present study, four human lung cancer cell lines were used; two squamous cell lines (QG-56, QG-95) and two adenocarcinoma cell lines (PC-9, PC-12). In all four tumor cell lines, mutations were detected in exon 7 of the p53 gene by a PCR-FSSCP analysis. The proliferation of QG-56 or QG-95 was inhibited by IFN-[gamma] in a dose-dependent manner with about 70% inhibition at 1000 JRU/ml while that of PC-9 was slightly inhibited with maximally 25% inhibition at 1000 JRU/ml. The growth of PC-12 was not inhibited at all. In QG-56, QG-95 and PC-9, the fraction of cells in G1 phase increased while the fractions of cells in both S and G2/M phases decreased after exposure to IFN-[gamma] (200 JRU/ml) for 72 h. The growth inhibition by long-term exposure to IFN-[gamma] was irreversible in QG-56. After culture in the presence of IFN-[gamma] (200 JRU/ml) for 14-16 days, tumor cells were examined for expression of various antigens, including HLA-class I, HLA-class II, and CEA. In all cell lines but PC-12, 100% of cells expressed HLA-class I after incubation with IFN-[gamma]. Both HLA-class II and CEA were also induced in those cell lines. The proportion of HLA-class II-positive cells or that of CEA-positive cells varied among the cell lines. Of the three antigens, the degree of HLA-class II expression paralleled that of growth inhibition by IFN-[gamma] treatment. These results suggested that in various function of IFN-[gamma] against tumor cells, the anti-proliferative effect might be closely linked with the induction of HLA-class II probably through a similar posttranscriptional process, independent of the function of p53 gene.

Loss of the G1 checkpoint appears to be extremely common among virtually all neoplasms. A variety of genetic and epigenetic mechanisms have been demonstrated to play significant roles in this process. In a consecutive series of early stage non-small cell lung cancer (NSCLC), we have established the loss of expression of the G1 Cdk inhibitors p15INK4b and p16INK4a by DNA methylation is very common (37%), and methylation of p16INK4a is tightly correlated with loss of expression of p16INK4a protein (P=0.0018). Furthermore, methylation of p15INK4b and p16INK4a appear inversely correlated, although methylation of p15INK4b is an infrequent event in this cohort (4%). Methylation was detected in all stages of NSCLC equally, and did not correlate with survival in these patients. Evidence for methylation was more frequent in squamous cell carcinomas in comparison to other tumor histologies (P=0.0156). In addition, over-expression of cyclin D1 was found to be tightly restricted (P=0.0032) to those tumors that had retained wild-type expression of pRB, and did not correlate with methylation or expression of p16INK4a gene product. Although loss of p16INK4a function remains tightly correlated with pRB expression, loss of other regulatory elements in NSCLC such as p53 mutation and cyclin D1 over-expression appear independent of loss of the p16INK4a gene product.

Mammalian Biology - Zeitschrift fur Saugetierkunde (1)


Native red deer of Mesola Wood, northern Italy, were compared with the Sardinian subspecies and with some populations from the Italian Alps and Austria using the analysis of restriction fragment length polymorphism (RELP) of mitochondrial (mt) DNA segments. The results highlight the existence of four main genetic lineages, and provide evidence for a structuring of populations according to their geographic occurrence. Two mitochondrial lineages, although highly distantly related, are shared between the populations from the centre-eastern Alps of Italy and Austria, while the other two lineages characterize the Sardinian and Mesola red deer, respectively. The exclusive haplotype found in the Mesola population appears as being an offshoot of one of the two main Alpine lineages, suggesting a presumed origin of these deer from a panmictic population which dwelt in mid-southern Europe, prior to the fragmentation of populations caused by human activities and manipulations. Considering their distinctiveness in morphologic and genetic traits, as well as their historical background and biogeographical value, these native deer should be regarded as a national conservation priority. The Sardinian red deer is highly divergent from both Mesola and Alpine populations. However, the controversial question of the phylogeographic origin of this subspecies remains unresolved. The utility of RELP analyses of mtDNA segments as a tool to discriminate among red deer populations as well as to develop effective strategies for their conservation and management.ZusammenfassungMitochondrien-DNA-Daten zur genetischen Unterscheidung der autochthonen Rothirsche von Mesola, Norditalien, von der Unterart auf Sardinien Autochthone Rothirsche aus dem Mesola-Waldgebiet in Norditalien wurden mittels Restriktionsfragmentenpolymorphismen (RFLPs) von Abschnitten der mitochondrialen (mt)DNA mit Rotwild der sardischen Unterart und Tieren aus alpinen Populationen verglichen. Die Ergebnisse weisen auf die Existenz von vier genetischen Hauptlinien hin und belegen auch eine Strukturierung der Populationen gem[a][beta] ihrer geographischen Herkunft. Zwei entfernt verwandte mtDNA-Linien wurden in Bestanden aus dem

Marine Pollution Bulletin (1)


http://www.sciencedirect.com/science/article/B6V6N-455TBHJ-K/2/594c31ff2db871eba8eb3af5940db305

Matrix Biology (15)


http://www.sciencedirect.com/science/article/B6VPM-44B213D-9/2/64a14940501849e3d4af5a8c8e8d5050

We have identified a novel 14-exon human lysyl oxidase-like gene, LOXL4, on chromosome 10q24. The cDNA and derived amino acid sequence of LOXL4 demonstrates a conserved C-terminal region including the characteristic copper-binding site, lysyl and tyrosyl residues and a cytokine receptor-like domain. One of the four N-terminal SRCR domains contains a 13 amino acid insertion encoded by a short exon not present within the closely homologous LOXL2 and LOXL3 genes. The 3.5-kb LOXL4 mRNA is present in pancreas and testis and at lower levels in several other tissues. Fibroblasts, smooth muscle and osteosarcoma (HOS) cells express LOXL4. No expression was detected in HCT-116 and DLD-1 colon, MCF-7 breast and DU-145 prostate cancer cell lines.

http://www.sciencedirect.com/science/article/B6VPM-41JTP8W-8/2/b7c91962c0e234eb8a9de9474362d01e

Pleiotrophin and chondromodulin-I are low molecular weight proteins that are abundant (20 [mu]g/g tissue) in fetal cartilage and difficult to detect in adult cartilage. We characterized their gene and protein expression patterns to gain a better understanding of their roles in the regulation of limb development and growth. In order to compare and contrast the relative amounts of the respective mRNA species within the developing epiphysis, a competitive PCR assay was developed. The results showed that the mRNAs for both proteins were abundant in fetal cartilage and while present in adult cartilage, were at 20-60-fold lower levels. Northern blotting revealed gradients of mRNA for both of these proteins in growth plate cartilage, with the highest levels in the resting zone, and the lowest in the hypertrophic zone. In contrast to pleiotrophin, chondromodulin-1 is down-regulated by retinoic acid with a pattern of expression similar to collagen type II and link protein, and may play a more specific role than pleiotrophin in modulating the chondrocyte phenotype.


http://www.sciencedirect.com/science/article/B6VPM-4BSW8KC-5/2/ce9b85ce63780f11e5fc5a99d1ca51a

Endoglin (CD105) is a homodimeric membrane glycoprotein, which acts as a TGF-[beta] coreceptor in the vasculature and plays an important role in cardiovascular development and vascular remodelling. To isolate putative genes regulated by endoglin expression, a PCR-based RNA fingerprinting technique was carried out. Myoblasts stably transfected with endoglin showed a decrease in the expression of lumican both at the RNA and protein levels. Lumican is a proteoglycan of the extracellular matrix, belonging to the SLRP (Small Leucine-Rich Repeat Proteoglycans) family. Lumican down-regulation by endoglin appeared to be controlled, at least in part, at the transcriptional level, as indicated by RT-PCR, and transient transfection experiments using a lumican promoter reporter based vector. This inverse correlation between endoglin and lumican expression was substantiated by immunohistochemical staining of vessels from human tissues. Thus, cells belonging to the high endothelia, such as tonsil, express a large amount of endoglin, and the lumican content of their matrix is considerably reduced. Conversely, in resting endothelia, such as that of large vessels, the expression of endoglin is reduced whereas the amount of lumican is greatly increased. The inverse regulation in the expression of endoglin and lumican was also evident after TGF-[beta] treatments since endoglin was up-regulated, whereas lumican was down-regulated by this cytokine. This report describes for the first time a relationship between endoglin and lumican expression.


http://www.sciencedirect.com/science/article/B6VPM-44PVT88-1/2/1dab29c60aa570ed11d39fc00c27f674

Ascorbic acid has been associated with the slowing of osteoarthritis progression in guinea pig
and man. The goal of this study was to evaluate transcriptional and translational regulation of cartilage matrix components by ascorbic acid. Guinea pig articular cartilage explants were grown in the presence of -ascorbic acid (-Asc), -isoascorbic acid (-Asc), sodium -ascorbate (Na -Asc), sodium -isoascorbate (Na -Asc), or ascorbyl-2-phosphate (A2P) to isolate and analyze the acidic and nutrient effects of ascorbic acid. Transcription of type II collagen, prolyl 4-hydroxylase (alpha subunit), and aggrecan increased in response to the antiscorbutic forms of ascorbic acid (-Asc, Na -Asc, and A2P) and was stereospecific to the -forms. Collagen and aggrecan synthesis also increased in response to the antiscorbutic forms but only in the absence of acidity. All ascorbic acid forms tended to increase oxidative damage over control. This was especially true for the non-nutrient -forms and the high dose -Asc. Finally, we investigated the ability of chondrocytes to express the newly described sodium-dependent vitamin C transporters (SVCTs). We identified transcripts for SVCT2 but not SVCT1 in guinea pig cartilage explants. This represents the first characterization of SVCTs in chondrocytes. This study confirms that ascorbic acid stimulates collagen synthesis and in addition modestly stimulates aggrecan synthesis. These effects are exerted at both transcriptional and post-transcriptional levels. The stereospecificity of these effects is consistent with chondrocyte expression of SVCT2, shown previously to transport -Asc more efficiently than -Asc. Therefore, this transporter may be the primary mechanism by which the -forms of ascorbic acid enter the chondrocyte to control matrix gene activity.


http://www.sciencedirect.com/science/article/B6VPM-40WDVPV-8/2/d2f1992f0b009049ad0caaa4773f63fc

Bovine joint capsule was maintained in explant culture in the presence of bovine aggrecan monomer and it was shown that the aggrecan monomer was degraded. Amino-terminal sequence analysis of the resulting aggrecan core protein fragments revealed that the core protein was cleaved at five specific sites attributed to glutamyl endopeptidases referred to as aggrecanase activity. Fibroblast cultures were established from explant cultures of joint capsule and when these cells were exposed to aggrecan, cleavage of the core protein of aggrecan at the aggrecanase sites was observed. Inclusion of either retinoic acid or interleukin-1[alpha] in medium of either joint capsule explant cultures or fibroblast cultures did not increase the rate of cleavage of exogenous aggrecan present in the culture medium. When aggrecan monomer was incubated with conditioned medium from explant cultures of joint capsule maintained in medium, degradation could be detected after 10 min. After a 6-h incubation period the same fragments of aggrecan core protein were observed as those for tissue or cells incubated directly with aggrecan monomer. RT-PCR analysis of mRNA extracted from joint capsule fibroblasts showed that these cells express both aggrecanase-1 and -2 [ADAMTS-2 (Tang) and ADAMTS-5].


http://www.sciencedirect.com/science/article/B6VPM-48N3JC5-2/2/09b5c6766bc243cfa7f1c7add918040b1

In the present study we investigated whether the collagen types I, III and V affect the activity of fibroblasts obtained from rabbit periosteum. The cells were cultured on plates either or not coated with different amounts of collagen type I, III or V and analyzed for their attachment, DNA synthesis and the expression and activity of matrix metalloproteinases (MMPs). Our data show that the three collagen types promoted attachment and spreading of the cells and stimulated DNA
synthesis when used in relatively low concentrations. High concentrations of type V—but not of type I or III—proved to inhibit thymidine incorporation. The expression and activity of matrix metalloproteinase 1 (MMP-1; interstitial collagenase) decreased under the influence of relatively low amounts of collagen (1-integrin or echistatin increased the level of MMP-1 but had no effect on MMP-2. All parameters tested were similarly affected by type I and III collagen, whereas the effect of type V was always less. We conclude that the collagen types I, III and V provide different sets of signals for fibroblasts that differently modulate their proliferation and MMP expression.


http://www.sciencedirect.com/science/article/B6VPM-46YXFH8-9/2/20e3abd241f27ab103ecef45e8742c53

Type IV collagenases/gelatinases (matrix metalloproteinases MMP-2 and MMP-9) in labial salivary glands (LSG) and saliva in Sjogren's syndrome (SS) and healthy controls were studied. Zymograms and Western blots disclosed that SS saliva contained 92/82 kD MMP-9/type IV collagenase duplex. Specific activity measurement disclosed 53.1 +/- 9.8 U/mg protein MMP-9 in SS compared to 16.5 +/- 2.6 U/mg in healthy controls (p = 0.01). MMP-2 did not differ between SS and controls. In SS salivary glands, MMP-2 and MMP-9 were also expressed, in addition to stromal fibroblasts and occasional infiltrating neutrophils, respectively, in acinar end piece cells. In addition, an effective proMMP-9 activator, human trypsin-2 (also known as tumor-associated trypsin-2 or TAT-2), was found in acinar end piece cells and in saliva. Interestingly, proteolytically processed MMP-9 was found in saliva (vide supra), and in vivo activated MMP-9 was significantly higher in SS than in controls (p = 0.002). LSGs, particularly in SS, were characterized ultrastructurally by areas containing small cytoplasmic vesicles in the basal parts of the epithelial cells associated with areas of disordered and thickened basal lamina. Based on our results, we conclude here that SS saliva contains increased concentrations of MMP-9, which is of glandular origin in part. Pro MMP-9 is to a large extent proteolytically activated. This is probably mediated by the most potent pro MMP-9 activator found in vivo thus far, namely trypsin-2. Therefore, the MMP-9/trypsin-2 cascade may be responsible for the increased remodelling and/or structural destruction of the basement membrane scaffolding in salivary glands in SS. Due to the role of basal lamina as an important molecular sieve and extracellular matrix-cell signal, these pathological changes may contribute to the pathogenesis of the syndrome.


http://www.sciencedirect.com/science/article/B6VPM-3Y3XR41-4/2/29b7d42200c08062426066ea150a7481

The human cDNA for cartilage intermediate layer protein (CILP) codes for a larger precursor protein that consists of CILP and a homologue to porcine Nucleotide pyrophosphohydrolase (NTPPHase) [Lorenzo et al. 1998a. J. Biol. Chem. 273, 23469-23475]. The human gene has now been isolated and characterized. Southern blot analysis indicated a single copy of the CILP gene in the human genome. The gene spans approximately 15.3 kbp of genomic DNA, and is organized in nine exons. The 5' flanking region contains a putative promoter region with a TATA-like box localized from -29 to -23 bp upstream of the transcription start site. Analysis of the putative promoter region revealed potentially cis-regulatory eukaryotic elements such as GATA-1, MyoD, MZF1, and CdxA. The protein coding region begins in exon 2 with the putative signal peptide. CILP is encoded from exon 3 to exon 9. In addition, exon 9 also codes for the entire NTPPHase homologue and contains the 3' untranslated region of the gene. All the introns follow
the ‘gt-ag’ rule, except the last intron, intron 8, that belongs to the minor class of pre-mRNA introns that contain ‘at-ac’ at their 5’ and 3’ ends, respectively. The CILP gene was mapped to human chromosome 15q22.


http://www.sciencedirect.com/science/article/B6VPM-44V2101-1/2/98d11c77df0765fe3767c67d2dd15ab0

We previously identified matrilin-2 (MATN2), the largest member of the novel family of matrilins. These filament-forming adapter proteins expressed in a distinct, but partially overlapping, pattern in all tissues were implicated in the organization of the extracellular matrix. Matrilin-2 functions in a great variety of tissues. Here, we present the genomic organization of the highly conserved mouse and human MATN2 loci, which cover >100 kb and 167.167 kb genomic regions, respectively, and are composed of 19 exons. RT-PCR analysis revealed that alternative transcripts with identical protein coding regions are transcribed from two promoters in both species. The upstream, housekeeping type promoter is functional in all tissues and cell types tested. The activity of the downstream, TATA-like promoter preceded with putative motifs for the homeobox transcription factor PRRX2 is restricted to embryonic fibroblasts and certain cell lines. The oligomerization module is split by an U12-type AT-AC intron found in conserved position in all four matrilin genes. We assigned Matn2 to mouse chromosome 15, linked to Trhr and Sntb1 in a region syntenic to human chromosome 8q22-24.


http://www.sciencedirect.com/science/article/B6VPM-4CPDFTP-5/2/e61391f65a1ed6f4c4943fa2fbebcb9

Matrilins are putative adaptor proteins of the extracellular matrix (ECM) which can form both collagen-dependent and collagen-independent filamentous networks. While all known matrilins (matrilin-1, -2, -3, and -4) are expressed in cartilage, only matrilin-2 and matrilin-4 are abundant in non-skeletal tissues. To clarify the biological role of matrilin-2, we have developed a matrilin-2-deficient mouse strain. Matrilin-2 null mice show no gross abnormalities during embryonic or adult development, are fertile, and have a normal lifespan. Histological and ultrastructural analyses indicate apparently normal structure of all organs and tissues where matrilin-2 is expressed. Although matrilin-2 co-localizes with matrilin-4 in many tissues, Northern hybridization, semiquantitative RT-PCR, immunohistochemistry and biochemical analysis reveal no significant alteration in the steady-state level of matrilin-4 expression in homozygous mutant mice. Immunostaining of wild-type and mutant skin samples indicate no detectable differences in the expression and deposition of matrilin-2 binding partners including collagen I, laminin-nidogen complexes, fibrillin-2 and fibronectin. In addition, electron microscopy reveals an intact basement membrane at the epidermal-dermal junction and normal organization of the dermal collagen fibrils in mutant skin. These data suggest that either matrilin-2 and matrilin-2-mediated matrix-matrix interactions are dispensable for proper ECM assembly and function, or that they are efficiently compensated by other matrix components including wild-type levels of matrilin-4.

http://www.sciencedirect.com/science/article/B6VPM-47T8XVT-3/2/09c76bc8fd8f57337779f517b6f9721

This study has examined the frequency and distribution of polymorphisms in the type 1 collagen coding sequences. RNA from a group of human skin fibroblast cell lines, was analyzed by the chemical cleavage mismatch detection method using hydroxylamine, a reagent specific for C base mismatches, and overlapping cDNA probes covering the entire prepro[alpha]1(I) and prepro[alpha]2(I) coding regions. Mismatches were detected at only two nucleotide positions, one in each of the type I collagen sequences, suggesting that polymorphisms are relatively rare within these cDNAs. cDNA sequence analysis demonstrated that the prepro[alpha]1(I) mismatch, detected in only one cell line, was due to a sequence polymorphism involving the wobble position of the codon for arginine residue 59 within the amino-propeptide globular subdomain of the pro[alpha]1(I) chain and not resulting in a change in the polypeptide primary structure. In contrast, the prepro[alpha]2(I) mismatch, detected in 6 of the 16 cell lines, was shown to arise from a sequence polymorphism affecting the identity of Y-position residue 459 of the [alpha]2(I) triple helical domain, resulting in an alanine/proline dimorphism at this position. This study is the first to identify a type I collagen coding sequence polymorphism resulting in an alteration at the level of the amino acid sequence. The data suggest that at least some [alpha]1(I) and [alpha]2(I) helix Y positions may be tolerant of sequence variation, particularly if the replacing amino acid is proline, a residue involved in stabilizing the collagen triple helix.


http://www.sciencedirect.com/science/article/B6VPM-47GH9YC-5/2/6d785b226dfcb2d2c6a39f04cef75db0

The coding regions of the human decorin, biglycan and fibromodulin cDNAs have been examined utilizing the method of single-strand conformation polymorphism analysis. Analysis of total RNA from a group of eight human skin fibroblast cell lines did not detect any sequence variations in the decorin cDNA. In contrast, the analysis detected three sequence variations in the biglycan cDNA and one in the fibromodulin cDNA from the same group of cell lines. For the biglycan cDNA, one variation involved a position in the 5'-untranslated region, while the other two affected the wobble bases of triplets encoding serine residues 10 and 143 of the mature core protein. For the fibromodulin cDNA, the variation involved the wobble position of the codon for glutamic acid residue 61 of the putative mature core protein. Single-strand conformation polymorphism analysis of these proteoglycan cDNAs was also applied to study patients exhibiting a variety of connective tissue pathologies, including chondrodysplasia punctata, Desbuquois syndrome, Dyggve-Melchior-Clausen syndrome, dyssegmental dysplasia, Ehlers-Danlos syndrome types I and III, Ellis van Creveld syndrome and thanatophoric dysplasia, though no additional sequence variations were detected.


http://www.sciencedirect.com/science/article/B6VPM-4F0GR57-1/2/b93bdf39d962c515c0244713b5e9d57
Lysyl hydroxylases 1, 2, and 3 catalyse the hydroxylation of specific lysines in collagen. A small percentage of these hydroxylysine residues are precursors for the cross-link formation essential for the tensile strength of collagen. Lysyl hydroxylase 2 (LH2) exists as two alternatively-spliced forms; the long transcript (the major ubiquitously-expressed form) includes a 63 bp exon (13A) that is spliced out in the short form (expressed, together with the long form, in human kidney, spleen, liver, and placenta). This study shows that this alternative splicing event can be regulated by both cell density and cycloheximide (CHX). Although only the long form of LH2 is detected in untreated confluent human skin fibroblasts, after 24 h treatment with CHX the short LH2 transcript is also expressed. In kidney cells, in which both LH2 transcripts are equally expressed, the long LH2 transcript is significantly decreased after 24 h CHX treatment, whereas expression of the short transcript is slightly increased. This suggests that, in kidney cells, the splicing mechanism for the inclusion of exon 13A in LH2 requires a newly-synthesized protein factor that is suppressed by CHX, whereas, in skin fibroblasts in which levels of LH2 (long) are unaffected, CHX appears to suppress a factor that inhibits exclusion of exon 13A, thereby promoting expression of LH2 (short). As these alternate transcripts of LH2 may have specificity for hydroxylation of lysines in either telopeptide or helical collagen domains, their relative expression determines the type of cross-links formed, thereby affecting collagen strength. Therefore, any perturbation of the regulation of LH2 splicing could influence the stability of the extracellular matrix and contribute to specific connective tissue disorders.


http://www.sciencedirect.com/science/article/B6VPM-47T2M7S-3/2/49c7a02a82d775d3b05fe4fe36e0b23

Versican is a large chondroitin sulfate proteoglycan (CSPG) initially identified in cultured human fibroblasts. Previous studies have shown that there is a versican-like molecule in cultured monkey smooth muscle cells. In this study, we have cloned and sequenced the large CSPG from cultured monkey smooth muscle cells, fetal and juvenile monkey aorta, and human fetal aorta. The cDNA sequence from human fetal aorta is completely homologous to the human fibroblast versican. We obtained 2.5 kb of cDNA sequence from monkey aortic RNA and cultured monkey smooth muscle cell RNA. This sequence covers three distinct domains of versican (hyaluronic acid binding domain, glycosaminoglycan attachment domain and protein binding domain) and demonstrates over 90% homology to the human versican sequence. In situ hybridization histochemistry indicates that the versican RNA transcript is located in the epithelium throughout the tunica media of the aorta. Western blot analysis and immunohistochemistry also confirm the presence of versican in human and monkey aorta.


http://www.sciencedirect.com/science/article/B6VPM-470KM47-4/2/52aa52578ad341bd4c2a9e7b974c6211

Previous studies suggested that remodeling of connective tissue is important in progression of atherosclerosis. We investigated the importance of matrix metalloproteinase 13 (MMP13), in the pathogenesis of atherosclerosis using 995 samples from the Pathobiological Determinants of Atherosclerosis in Youth collection in an association study. We identified two new MMP13 promoter polymorphisms. The genotype for one of the MMP13 polymorphisms was associated with fibrous plaque (P=0.024) in black males. Immunohistochemistry using antibodies for MMP13
showed that MMP13 is expressed in all layers of the aorta. In-vitro transfection experiments with reporter gene constructs and electrophoretic mobility-shift assays showed that the MMP13 polymorphism was a functional variant. MMP13 is therefore, a genetic risk factor for extent of fibrous plaque in the abdominal aorta in young black males. Elucidation of the currently unknown mechanism of the MMP13 polymorphism's action may provide for pharmacological intervention to reduce the severity of atherosclerotic changes in susceptible individuals.

Maturitas  (5)

http://www.sciencedirect.com/science/article/B6T9F-4DF4D3G-1/2/0bed3adc5adb1ce693b7eda24819ba76

Objectives: Osteoprotegerin (OPG) is a recently discovered member of the tumour necrosis factor receptor superfamily. It plays a crucial role in the control of bone resorption and its gene could therefore be a good candidate gene for osteoporosis. The aim of our work was to find polymorphisms in the OPG gene and to investigate their possible contribution to the genetic susceptibility to osteoporosis by testing for their association with bone mineral density (BMD).

Methods: The whole OPG gene coding region was screened for the presence of polymorphisms in a group of 60 osteoporotic women by single-strand conformation polymorphism analysis (SSCP) approach. Association of the discovered polymorphisms with bone mineral density was investigated in 136 Slovenian postmenopausal women.

Results: We detected eight OPG gene polymorphisms that were confirmed by direct DNA sequencing, deletion 4752_4753delCT and nucleotide substitutions 1181G > C, 1217C > T, 1284G > A, 4501C > T, 6893A > G, 6950A > C and 8738T > A. Nucleotide substitutions 1284G > A and 8738T > A have not been previously described. Polymorphisms 4752_4753delCT, 6893A > G and 6950A > C were in complete linkage and the same was true for 1217C > T and 4501C > T. The association with BMD was found only for polymorphism 1181G > C. Subjects with genotype 1181GG had significantly lower lumbar spine BMD than subjects displaying 1181GC.

Conclusions: By our approach we detected eight polymorphisms in the OPG gene. According to our analysis polymorphism 1181G > C is associated with BMD and could therefore be considered as an element of genetic susceptibility to osteoporosis.

http://www.sciencedirect.com/science/article/B6T9F-44F6SB1-4/2/43554eca3c2a947ad222558c6a6a90a

Objective: Osteoporosis is a common disorder with a strong genetic component. Our aim was to investigate the correlation of the estrogen receptor [alpha] gene microsatellite polymorphism (TA dinucleotide repeat polymorphism 5’ upstream of exon 1) with bone mineral density and their relationship to osteoporosis. Methods: We determined the estrogen receptor [alpha] gene microsatellite polymorphism using polymerase chain reaction-based microsatellite analysis in postmenopausal Chinese women in Taiwan. Bone mineral density of the lumbar spine and
proximal femur were measured using dual-energy X-ray absorptiometry. Results: The ER[alpha] genotype was classified into '12' through '27' according to the number of TA dinucleotide repeats they contained, as a 'signpost'. After adjustment for potential confounding factors including age, height, and weight, subjects with genotype 18+ (n=4) had lower bone mineral density values and a 54.5 times greater risk for osteoporosis when compared with subjects with genotype 18- (n=170) at the lumbar spine. This should be interpreted with caution because of the small number of subjects with the unfavorable genotype 18+. According to mean number of TA dinucleotide repeats, women with a high number of repeats (TA[GE]20) (n=38) had the lowest bone mineral density and a 6.1 times greater risk for osteoporosis than women with a low number of repeats (TA[LE]15) (n=61) at the femoral neck, after adjustment for potential confounding factors such as age, height, and weight. Conclusion: The present study suggests that the estrogen receptor [alpha] gene microsatellite polymorphism may be a candidate genetic marker for risk of osteoporosis in postmenopausal Chinese women in Taiwan.


http://www.sciencedirect.com/science/article/B6T9F-47FDMSJ-2/2/b48f6a5d87fafa3f76308ea0ba64999eb6

Objective: Osteoporosis is a common disorder with a strong genetic component. Our aim was to investigate the correlations of the interleukin-1[beta] (IL-1[beta]) and interleukin-1 receptor antagonist (IL-1(Ra)) gene polymorphisms with bone mineral density (BMD) and their relationship to osteoporosis. Methods: The IL-1[beta] (promoter and exon 5) and IL-1(Ra) (intron 2) gene polymorphisms were determined using polymerase chain reaction. BMD of the lumbar spine and proximal femur were measured using dual-energy X-ray absorptiometry. Results: The prevalence of each genotype of the interleukin-1 related genes in the study population was: (1) 14% C/C, 71.5% C/T, and 14.5% T/T in IL-1[beta] promoter; (2) 95.3% E1/E1 and 4.7% E1/E2 in IL-1[beta] exon 5; (3) 92.4% I/I, 6.4% I/II, and 1.2% II/II in IL-1(Ra) intron 2. After adjustment for potential confounding factors such as age, height, weight, years since menopause, and daily calcium intake, subjects with genotype E1/E2 (n=8) in IL-1[beta] exon 5 had lower BMD values and a significantly greater risk for osteoporosis (OR 10.6, 95% CI 1.3-83.8) at the lumbar spine when compared with subjects with genotype E1/E1 (n=164) in IL-1[beta] exon 5. Conclusion: The Taq I IL-1[beta] exon 5 gene polymorphism is associated with reduced BMD and predisposes women to osteoporosis at the lumbar spine, but our results should be interpreted with caution because of the small number of subjects with the unfavorable E1/E2 genotype.


http://www.sciencedirect.com/science/article/B6T9F-4D7K6Y9-1/2/c3bd3a050e898a062cb3eeced473d507b

Objectives: The aim of this study was to examine the expression of oestrogen regulated genes in premenopausal and postmenopausal normal and malignant endometrial specimens. The molecular mechanisms and the role of these genes in endometrial carcinogenesis are poorly understood. Methods: Normal and malignant endometrial specimens were collected from patients undergoing hysterectomy. Real time TaqMan PCR was used to examine the mRNA expression levels of oestrogen receptor a (ERa) and b (ERb), progesterone receptor (PR), insulin like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). Results: Expression analysis was carried out on 60 patients. ERa was more predominantly expressed in the endometrial samples than ERb, 28% of the specimens did not express ER. Normal pre and postmenopausal tissue
expressed higher levels of ERa, PR and IGF-1 than malignant tissue. ERa and PR expression was significantly higher in the proliferative phase endometrium compared to the secretory phase (P Conclusions: ERa expression may play an important role in the regulation of PR in normal and malignant endometrium. Further work is needed to establish if IGF-1 plays a role in a subset of endometrial cancers and if isoforms of VEGF play a role in endometrial cancer.


Estradiol (E2) and testosterone (T) effectively prevent orchidectomy (orx) induced osteoporosis. T, however, stimulates prostate proliferation which may lead to malignancy. We showed that a Cimicifuga racemosa (CR) preparation had bone-sparing effects without exerting estrogenic effects in the uterus. We studied therefore whether a CR preparation has also antosteoporotic effects in orx rats substituted with E2, T or CR via pelleted food over a period of 3 months. Average daily intake per animal was: T: 25 mg; E2: 0.325 mg, CR low dose: 33 mg; CR high dose: 133 mg. E2, T and CR at the high dose partially prevented development of osteoporosis as measured by quantitative computer tomography in the metaphysis of the tibia. E2, but not T or CR reduced serum osteocalcin and the metabolic products of collagen-1[alpha]1. Gene expression of collagen-1[alpha]1 and tartrate-resistant acid phosphatase was decreased by E2 and the higher dose of the CR extract but increased in the T-treated animals. In the prostate T inhibited androgen receptor, estrogen receptor [alpha] and insulin-like growth factor-1 gene expression but stimulated the expression of the ER[beta] gene. These effects were not shared by E2 or both doses of the CR extract. It is concluded that E2, T and CR exert antosteoporotic effects in the metaphysis of the tibia of orx rats. T has profound effects in the prostate which were not seen in the E2- and CR-treated animals. Therefore, the Cimicifuga racemosa extract BNO 1055 may be useful to prevent osteoporosis in aged male patients with reduced testosterone production.

Meat Science (2)


http://www.sciencedirect.com/science/article/B6T9G-4CHHR4K-8/2/af96ed0f5d3c28e9e57197abb8bad8

Species-specific real-time PCR (TaqMan) assays were developed for detection of beef, pork, lamb, chicken and turkey. Assays were developed around small (amplicons b (cytb) gene. Speciation was achieved using species-specific primers. For detection purposes, two TaqMan probes were developed; the first was specific to the mammalian species (beef, lamb and pork), the second to the poultry species (chicken and turkey). Normal end-point TaqMan PCR conditions were applied; however, PCR was limited to 30 cycles. Applying the assays to DNA extracts from raw meat admixtures, it was possible to detect each species when spiked in any other species at a 0.5% level. The absolute level of detection, for each species, was not determined; however, experimentally determined limits for beef, lamb and turkey were below


A rapid and highly specific assay suitable for the routine detection of turkey and chicken in processed meat products has been developed. Based on PCR amplification of species-specific amplicons with rapid visualisation using vistra green, the assay may be completed within 5 h of receipt of sample. DNA was isolated from meat samples by the use of Wizard DNA isolation technology and followed by DNA amplification in the polymerase chain reaction using species specific primers, chicken forward (CF), chicken reverse (CR), turkey forward (TF) and turkey reverse (TR): the production of an amplicon was detected after the end of the PCR in less than 5 min using vistra green and a fluorescence plate reader. The presence of fluorescence denoted the presence of the target species in the sample.

**Mechanisms of Ageing and Development** (12)


http://www.sciencedirect.com/science/article/B6T31-4031VR5-5/2/288ee226303e28d562da033ec310a6cd

Alloantigen stimulation was used to examine the effect of interleukin (IL-12) treatment of stimulated cells from young and aged mice on the expression of mRNAs for perforin and granzyme B, two proteins known to be intimately involved in an important lytic pathway used by CTL, and mRNA for interferon (IFN)-[gamma], production of which is highly stimulated by IL-12. As reported previously, IL-12 augmented the lytic activity by cells from both young and aged mice, although the relative increase was greater for the latter. The mRNAs encoding perforin and granzyme B were both marginally enhanced at early time points (for cells from young mice) or throughout the stimulation (for cells from aged mice) following allo-stimulation in the presence of IL-12. The levels of augmentation of these mRNAs was consistent with the augmentation of lytic activity. In contrast, mRNA encoding IFN-[gamma] was markedly enhanced throughout stimulation in cells from animals of both age groups, corresponding to the more substantial increase in interferon protein in response to IL-12.

We investigated the production of IL-2 and IFN-\([\gamma]\) (Th1 type) and IL-4 (Th2 type) cytokines by mitogen-activated spleen cells from young, adult and old mice. Cytokine production was evaluated in culture supernatants by CTLL proliferation (IL-2), ELISA (IFN-\([\gamma]\)), CT4.S proliferation (IL-4) and in mRNA extracted from activated CD4+ cells by RT-PCR (IL-2, IFN-\([\gamma]\) and IL-4). Results show that the production of IL-2, as protein and mRNA, is profoundly depressed by aging, whereas that of IFN-\([\gamma]\), as protein and mRNA, firstly declines and then increases with age. The production of IL-4, as protein, monotonically declines with aging whereas, as mRNA, firstly decreases and then increases above the level in young mice. Spleen cells in culture were also incubated with mitogens and with a recombinant cytokine (IL-1\([\beta]\), IL-2, IL-3, IL-4, IL-12 or IFN-\([\gamma]\)) at various concentrations. It was found that recombinant cytokines by and large enhance cytokine production when the level induced by mitogens only is low. This conclusion applies to IL-2 and IFN-\([\gamma]\) production as protein and mRNA. The addition of recombinant cytokines also increases the production of IL-4 at the protein level in spleen cells from old mice but, at the mRNA level, only in spleen cells from young mice. This finding suggests age-related changes in IL-4-specific mRNA transcription rate and post-transcriptional half-life as well as translation kinetics.


http://www.sciencedirect.com/science/article/B6T31-3SY3GPDP-6i2/883d566bc38f272e91bf524c8a5bf326

It is clear that there is a genetic component associated with the ageing process. Although evolutionary theory has suggested that the activity of certain genes may facilitate ageing by favouring resource utilisation by the germ cells at the expense of somatic cells, there is reason to believe that the senescent phenotype, which is the endpoint of the ageing process, may be due to alterations in the levels of expression of other genes. To investigate this situation we have used the differential display technique to survey gene expression during ageing of the rat brain, heart and liver. By optimising this technique it is possible to identify up to 10000-14000 PCR products, which represent genes expressed in the tissue under study. Interestingly, only a relatively small cohort ([ap]2%) of these genes appear to show significant changes in their levels of expression during ageing. Characterisation of the latter has so far revealed certain genes, such as glial fibrillary acidic protein, which are associated with the senescent phenotype. It has also revealed that the level of fos, a component of the AP-1 transcription factor, decreases with age, which has implications for AP-1 regulated genes. The differential display technique has also revealed an increase in mitochondrial RNA during ageing of the heart, which may be due to a gene dosage effect caused by the presence of increased numbers of mitochondrial genomes in myocytes in old age. The differential display technique therefore appears to offer a powerful tool for identifying genes which contribute to the emergence of a senescent phenotype.


http://www.sciencedirect.com/science/article/B6T31-3SBVW1W-72/a25302ff79b2094b024744160b6d7978
The sinoatrial (SA) node is the cardiac pacemaker and changes in its adrenergic-muscarinic phenotype have been postulated as a determinant of age-associated modifications in heart rate variability. To address this question, right atria were microdissected, the SA node area was identified by acetylcholinesterase staining, and, using a RT-PCR method, the accumulation of mRNA molecules encoding [beta]1- and [beta]2-adrenergic ([beta]1- and [beta]2-AR) and muscarinic (M2-R) receptor was quantified to define the proportion between [beta]-AR and M2-R mRNAs within the sinoatrial area of adult (3 months) and senescent (24 months) individual rat hearts. 

In adult hearts, the highest M2-R/[beta]-AR mRNA ratio was observed within the sinoatrial area compared with adjacent atrial myocardium, while in the senescent hearts, no difference was observed between sinoatrial and adjacent areas. This change was specific of the sinoatrial area since adult and senescent whole atrial or ventricular myocardium did not differ in their M2-R/[beta]-AR mRNA ratio, and was associated with a fragmentation of acetylcholinesterase staining of the senescent SA node. Quantitative changes in the expression of genes encoding proteins involved in heart rate regulation specifically affect the sinoatrial area of the senescent heart.


We used a fluorescence differential display -- PCR (FDD-PCR) technique to analyze the genes expressed in mouse kidneys collected at nine different developmental stages ranging from 3 days to 15 months after birth. We found ten genes that were age-dependent and differentially-expressed in the kidneys during our experimental period. We confirmed by comparative RT-PCR that of the ten cDNAs, seven showed reproducible age-dependent expression. Four of the nucleotide sequences of these cDNA clones, had high homology with known genes (fibronectin, soluble guanylyl cyclase [alpha]-1 subunit, cytosolic aldehyde dehydrogenase and mitochondrial DNA), and three with expressed sequence tags of unknown genes. The FDD-PCR method was very useful for detecting new age-related genes expressed differentially in the mouse kidney.


Altered calcium homeostasis in the senescent heart appears to be the result, at least in part, of decreased Na+/Ca2+ exchange activity. To further investigate the basis of the decrease in Na+/Ca2+ exchange activity, Na+/Ca2+ exchanger gene expression in the heart was compared in 3 and 24 month old male Fischer 344 rats. Sarcolemmal vesicles prepared from left ventricle and septum showed reduced Na+-dependent Ca2+ uptake in 24 month old animals when compared to 3 month old animals (0.156 +/- 0.005 and 0.135 +/- 0.008 nmol Ca2+/mg/10 s; mean +/- S.E. for 3 month and 24 month old animals, respectively). Western analysis showed immunodetectable Na+/Ca2+ exchanger protein levels were decreased by 19% in 24 month old animals when compared to 3 month old animals. Poly(A+) RNA was purified from left and right ventricle and left and right atria and subjected to Northern analysis using digoxin labeled cDNA probes for the Na+/Ca2+ exchanger and actin. The Na+/Ca2+ exchanger probe labeled a 7 kb message in both ventricle and atria, while the actin probe labeled both [beta]-actin (2.2 kb) and [alpha]-actin (1.4 kb). The steady state level of expression of Na+/Ca2+ exchanger Poly(A+) RNA when normalized to [beta]-actin, was similar when ventricle and atria were compared. There were
no observable differences in Na+/Ca2+ exchanger or [alpha]-actin Poly(A+) RNA steady state levels when comparing 3 and 24 month old animals. The results suggest that reduced Na+/Ca2+ exchange activity in the left ventricle of 24 month old animals was most likely the result of post-transcriptional modification of the protein that was detectable by Western analysis.

Jung, K.-Y., D. Dean, et al. (2004). "Loss of N-cadherin and [alpha]-catenin in the proximal tubules of aging male Fischer 344 rats." 

http://www.sciencedirect.com/science/article/B6T31-4C8DD85-1/2/f64e1fcd3b3c2f00a4b40a6ae1b44c26

Aging is associated with a loss of renal reserve, and increased sensitivity to either xenobiotic or physiologic insult. Given the critical role of the cadherin/catenin complex in establishing and maintaining the integrity and polarity of tubular epithelial cells, it was hypothesized that aging was associated with alterations in renal cadherin/catenin complexes. Histological assessment of aged (24 months) kidneys harvested from male Fischer 344 rats demonstrates mild degeneration of proximal tubules, multifocal chronic lymphocytic infiltration, moderate development of protein casts inside tubules, and tubular dilatation or degeneration. Western blot analysis revealed that N-cadherin protein expression is not constant over 24 months. N-cadherin expression increased from 4 to 9 months, with peak levels at 9 and 13 months. A decrease in expression was seen at 19 months and an almost complete loss of expression was seen at 24 months. In contrast, the expression of E- and Ksp-cadherin was constant over 24 months. A loss of [alpha]-catenin at was seen at 19 and 24 months in the absence of changes in [beta]-, [gamma]-, and p120-catenin. This pattern of N-cadherin expression (increase followed by decrease) was confirmed by real-time PCR analysis, which demonstrated a similar pattern as the Western blot, suggesting that the loss of N-cadherin protein was due to decreased gene expression. The loss of N-cadherin was specific for the kidney, as no changes in N-cadherin expression in the liver, brain, or testes were seen during aging. The conclusion that loss of N-cadherin expression is a critical component of the renal dysfunction associated with aging is supported by the finding that caloric restriction attenuates the loss of N-cadherin, as well as the finding that a significant loss of N-cadherin is seen in the kidneys of ZDF x SHHF rats, a genetic model of end-stage renal disease. Cadherin and catenin expression was further analyzed by immunofluorescence. A significant loss of staining of both N-cadherin and [alpha]-catenin was seen in the proximal tubules of rats at 24 months. Interestingly, this corresponded with delocalization of the [alpha]-1 subunit of the Na+K+-ATPase, i.e. aberrant staining on cell-cell borders and some indication of apical staining in proximal tubules. Taken together, these data suggest that aging is associated with decreased expression of N-cadherin and [alpha]-catenin and is associated with a loss of cell polarity.


NMDA receptors play an important role in memory processes and plasticity in the brain. We have previously demonstrated a significant decrease in NMDAR[epsiv]2 subunit mRNA and protein with increasing age in the C57Bl/6 mouse frontal cortex. In the present study, two-electrode voltage clamp electrophysiology on Xenopus oocytes injected with total RNA harvested from the frontal cortex of young and old C57Bl mice was used to detect changes in receptor composition during aging. Ifenprodil concentration-response curves, magnesium current-voltage curves, and single channel conductances were determined for native receptors. In addition, ifenprodil and
magnesium curves were generated for recombinant NMDA receptors of varying subunit ratios. Ifenprodil dose-response curves for all receptors were biphasic. The low affinity component of the curve increased slightly with age, while the high affinity population decreased, mimicking recombinant receptors with decreasing levels of [epsiv]2. A decrease in maximal current was also observed in aged animals with decreased levels of [epsiv]2, although single channel conductances were identical between young and old mice. In addition, an increase in sensitivity to magnesium was observed for receptors from older animals. Results are consistent with the interpretation that the [epsiv]2 subunit is reduced in older mouse frontal cortex. A change in NMDA receptor subunit composition could influence memory processes during aging.


http://www.sciencedirect.com/science/article/B6T31-41F62WP-4/2/dbd2b07103f96fe873b2355545c0b2f7

Studies of the frequencies of different alleles in young adults and aged individuals have implicated several genes, such as ApoE and ACE, in longevity. However such association studies can easily give rise to spurious results through unsuspected population subdivision, and an approach making use of genetic relationships among relatives is desirable. We have studied the effectiveness of non-parametric genetic analysis to detect different types of loci affecting longevity. The non-parametric method has high statistical power to detect infrequent recessive alleles that are required for, or significantly increase the probability of, survival to advanced age. Statistical power is reduced if a proportion of carriers of the alternative allele is allowed to survive. The method is least effective in detecting alleles that occur at low frequency in young individuals and that subsequently experience high mortality, as is the case for carriers of the [epsiv]4 allele of ApoE. Genotyping errors will also reduce the value of the NPL statistic in a linear fashion with the error rate and the number of loci genotyped. We have also used the method to analyse genotypes of seven highly polymorphic markers near the ApoE gene in a sample of 188 sibships of nonagenarians and centenarians (n=434) and their children (n=124), however no excess sharing of alleles was detected.


http://www.sciencedirect.com/science/article/B6T31-3SY3GPD-4/2/a543014d6fe9a83108ad0b1174a4aa5e

Hutchinson-Gilford progeria syndrome (HGPS) is a fatal segmental aging disorder affecting children. There is a paucity of prior data at the nucleotide level on DNA maintenance in HGPS. We have examined the specific nucleotide sequences and production of allelic transcripts from the locus GGTB2 encoding [beta](1-4) galactosyltransferase. Quantitative Northern blots of mRNA from HGPS and control fibroblasts indicated identical mature [beta](1-4) galactosyltransferase transcript sizes and amounts, regardless of their altered glycosylation status. DNA sequencing of cDNA derived from HGPS [beta](1-4) galactosyltransferase mRNA populations confirmed the encoded amino acid sequence was unaffected. Population studies of 41 unrelated individuals provided allelic frequency estimates for a novel FokI polymorphism, which was identified in two of six progeria cell strains. The polymorphism was faithfully inherited in a progeria pedigree in a Mendelian manner. Furthermore, the polymorphism provided direct evidence through sequencing of reverse transcription polymerase chain reaction products that
both alleles were transcribed and generated mature mRNA. Any defects in transcripts were below detectable levels over the lengths of coding sequences examined, despite multiple replication events from conception leading to the production and maintenance of patient-derived cells. These results indicate faithful transcription in HGPS.


http://www.sciencedirect.com/science/article/B6T31-3WHKRFF-5/2/9d39ccaac1423ba891584062951de9a3

The production of prostaglandin (PGE2) in human periodontal ligament fibroblast (hPLF) cells is increased by mechanical stress, however, the age-related changes in the susceptibility of hPLF cells in response to mechanical stress remain unclear. The purpose of this study was to examine the influence of in vitro cellular aging on PGE2 production and the gene expression of cyclooxygenase (COX) in mechanically stressed hPLF cells. In vitro cellular aged hPLF cells were prepared by sequential subcultivations of hPLF cells from young healthy periodontal ligaments. In vitro cellular aged hPLF cells produced a significantly higher amount of PGE2, as compared with young hPLF cells, when the cells were exposed to cyclic tension force in a time- and magnitude-dependent manner. The COX-2 mRNA level in aged cells was higher than that in young cells, whereas COX-1 mRNA remained unchanged. Since PGE2 from hPLF cells was stimulated by in vitro aging as presented here, aging of hPLF cells may affect the severity of inflammation and bone resorption in the aged through the production of a large amount of PGE2 in response to an excessive force such as a traumatic occlusion.


http://www.sciencedirect.com/science/article/B6T31-47F750C-1/2/fabed59b1c3d8a624db946ccc0d5bb52

To explore new models for human cellular aging as well as to evaluate aging of the macaques, profiles of cellular aging in macaques were studied. Adherent cells were obtained from five Japanese macaques (Macaca fuscata), 14 long-tailed macaques (Macaca fascicularis), two bonnet monkeys (Macaca radiata) and a rhesus monkey (Macaca mulatta). A total of 35 cultures were performed and cell morphology, doubling time, telomere length and telomerase activity were studied. They were classified into three groups: group I: cell strains with a definite replicative life-span (<41 PDLs) (presence of M1), group II: cell strains with a limited extension of replicative life-span (79-106 PDLs) with p53 mutation(s) (presence of M2), and group III: a cell strain with an indefinite replicative life-span (>150 PDLs) with characteristics of transformation. Except for the last group, telomerase activity was not observed. Macaque cells demonstrated three chronological patterns comprising both human and rodent patterns, however, presence of the two limits of proliferation in vitro grants macaque cells to be more appropriate than rodents in both studying human aging and oncogenesis.

http://www.sciencedirect.com/science/article/B6T9H-43VSC56-1/2/4ed51d02554b38d46c8b5c9ebc938efd

Many studies have suggested that transforming growth factor [beta] (TGF-[beta]) and bone morphogenetic protein 4 (Bmp4) regulate early development of the lung. In this study, administration of growth factors directly into the lumen of lungs grown in organ culture was used to limit their activity to the epithelium and test the hypothesis that signaling to the epithelium is sufficient to mediate the known effects of TGF-[beta] and BMP-4 on early lung development. Addition of TGF-[beta]1, [beta]2, or [beta]3 to the medium surrounding lungs grown in organ culture resulted in decreased branching, reduced cell proliferation, accumulation of [alpha]-smooth muscle actin protein ([alpha]-SMA) in the mesenchyme, and decreased expression of a marker for respiratory epithelium, surfactant protein-C (Sp-C). When TGF-[beta]1 was restricted to the epithelium, accumulation of [alpha]-SMA and inhibition of Sp-C expression were not observed but branching and proliferation were inhibited. In contrast, branching was not inhibited in lungs where TGF-[beta]2 or TGF-[beta]3 were restricted to the epithelium suggesting differences in the mechanism of signaling by TGF-[beta]1, TGF-[beta]2 or TGF-[beta]3 in lung. Addition of Bmp4 to the medium surrounding lungs grown in organ culture stimulated cell proliferation and branching morphogenesis; however, direct injection of Bmp4 into the lung lumen had no effect on proliferation or branching. Based on these data and data from mesenchyme-free cultures, we propose that the mesenchyme influences growth factor signaling in the lung.


http://www.sciencedirect.com/science/article/B6T9H-40D61D7-G/2/244cdf18fa8047c379b379b96ea5bc8337e7

We characterized a Pax gene from the hydrozoan Podocoryne carnea. It is most similar to cnidarian Pax-B genes and encodes a paired domain, a homeodomain and an octapeptide. Expression analysis demonstrates the presence of Pax-B transcripts in eggs, the ectoderm of the planula larva and in a few scattered cells in the apical polyp ectoderm. In developing and mature medusae, Pax-B is localized in particular endodermal cells, oriented toward the outside. Pax-B is not expressed in muscle cells. However, if isolated striated muscle tissue is activated for transdifferentiation, the gene is expressed within 1 h, before new cell types, such as smooth muscle and nerve cells, have formed. The expression data indicate that Pax-B is involved in nerve cell differentiation.


http://www.sciencedirect.com/science/article/B6T9H-49H1FN9-1/2/4f079e3f8aa2d08249e03ff39ce3f7da

The sea urchin orthodenticle (Otx)-related transcription factor is an early activator of other
endomesodermally expressed transcription factors. Its normal function is required for the development of the archenteron and to lock cells into endomesodermal fate. To determine if this is a basal Otx function in echinoderms we have studied the role of an Otx ortholog in a starfish, Asterina miniata. The patterns of AmOtx expression are found to be similar, in many details, to those reported for other indirectly developing echinoderms and hemichordates, suggestive of a conserved function both in endoderm development and ciliary band formation in these two phyla. When downstream targets of the AmOtx protein are suppressed using a dominant engrailed repressor strategy, embryos fail to develop the endodermal component of the archenteron, though initial phases of mesoderm development proceed normally. The function of Otx proteins in endodermal development at least predated the evolution of the free-living echinoderms (Eleutherozoa).


Using a Drosophila cell-free system, we have analyzed the regulation of alternative splicing of Drosophila muscle myosin heavy chain (MHC) transcripts. Splicing of MHC 3’ end transcripts results in exclusion of adult-specific alternative exon 18, as is observed in embryonic and larval muscle in vivo. Mutations that strengthen either the 5’ or the 3’ splice sites of exon 18 do not promote inclusion of this exon. However, strengthening both splice junctions results in efficient removal of both introns and completely inhibits skip splicing. Our data suggest that the affinity of exons 17 and 19, as well as failure of constitutive splicing factors to recognize exon 18 splice sites, causes the exclusion of exon 18 in wild-type transcripts processed in vitro.


Nuclear orphan receptors are DNA binding proteins that share the domain structure of the nuclear hormone receptor superfamily, although ligands are unknown. We have identified an orphan receptor in Xenopus laevis and named it xGCNF based on its high degree of sequence homology to the previously described murine germ cell nuclear factor (mGCNF). In gel-electrophoresis mobility shift analysis experiments in vitro translated xGCNF and mGCNF proteins both bind specifically as homodimers to the same response element, a direct repeat of the half-site consensus AGGTCA with zero spacing (DR0). Transcripts of xGCNF are found in oocytes and in much smaller amounts in the testes. In developmental Northern blots and RNase protection using RNA from different embryonic stages, zygotic expression of xGCNF peaks at midneurula. From late gastrula to midneurula stages, an anterior to posterior concentration gradient of the RNA was observed in whole mount in situ analysis. This antero-posterior gradient of expression was also observed in exogastrulae, both in the ectoderm and mesoderm. In the midneurula embryo, the mRNA was predominantly found in the neural plate and neural crest. Transcription of xGCNF in animal cap explants occurred independent of mesoderm induction.
In zebrafish, maternally produced vasa (vas) transcripts become targeted to the cleavage planes of early embryos and subsequently incorporated into the primordial germ cells (PGCs). Zygotic vas transcription occurs from the onset of gastrulation. Here, we report on the characterisation of the zebrafish vas locus. The gene consists of 27 exons, spans about 25 kb, and contains two CpG-rich regions. We have used vas regulatory regions to establish transgenic zebrafish lines expressing enhanced green fluorescent protein (EGFP) in their PGCs. Maternally encoded vas:EGFP transcripts and VAS:EGFP protein segregate with the PGCs during embryogenesis. We find that the maternally deposited vas:EGFP transcripts are stable during embryogenesis at least up to 50 h of development. Vas:EGFP transcripts could not be detected in embryos that inherit the transgene from males, most likely due to the lack of one or more regulatory elements required for early zygotic expression. We show that vas:EGFP transcripts become enriched to the cleavage planes in early embryos, a finding that supported an RNA localisation signal localised within the vas region of these transcripts.

A Drosophila gene encoding a novel zinc-finger protein, Meics, was cloned using a monoclonal antibody. The predicted amino acid sequence contains 12 zinc-finger motifs of the C2H2-type. During spermatogenesis, Meics distributes intranuclearly at pre- and post-meiotic stages whereas it relocates to central-spindle microtubules at both meiotic divisions.

The POU transcription factor Oct-4 is expressed in early mouse embryogenesis and in pluripotent embryonal stem (ES) and embryonal carcinoma (EC) stem cell lines. After gastrulation in the embryo, Oct-4 expression is confined to the germline. The present study provides evidence that Oct-4 undergoes downregulation during oogenesis and spermatogenesis, coincident with entry into meiosis. Furthermore, analysis of maturation stages of oocytes showed that Oct-4 is upregulated de novo in the final stages of meiotic prophase I in female germ cells. These data suggest that Oct-4 downregulation in germ cells in both sexes might represent one of the molecular triggers involved in the commitment to meiosis. The upregulation of Oct-4 in oocytes at the completion of the prophase I of meiotic division further suggests a specific involvement of this transcription factor in oocyte growth or the acquisition of meiotic competence.

http://www.sciencedirect.com/science/article/B6T9H-442HVW8-7/2/03317bce14226ec767e32a210f8f35bd

We describe the cloning of HOXD1 in human unfertilised oocytes and detailed expression analyses during mouse oogenesis and embryogenesis. The cDNA of 1991 bp has an open reading frame of 987 bp encoding a protein of 329 amino acids. A comparison of the amino acid sequence with the mouse homologue revealed an overall homology of 85.5% with 99% identity within the homeodomain. Expression was detected in unfertilised human oocytes and 2-, 4-, 8-cell and blastocyst stage embryos. Expression analyses in mature mouse ovaries, early embryos and isolated gut revealed expression in the oocytes of the primary and secondary ovarian follicles, and in embryonal mesodermal derivatives such as dermatomes, urogenital tubercle, tail bud, kidney, ovaries, testes and enteric mesoderm adjacent to the caecum where expression was up-regulated in vitro in response to increasing doses of retinoic acid. Our observations indicate a possible role for HOXD1/Hoxd1 in the ovarian oocytes and the establishment of mesodermal derivatives during embryogenesis.


http://www.sciencedirect.com/science/article/B6T9H-3YVM6H9-C/2/e25af882159dbec2fc406420442d1f18

We report that activin profoundly alters epithelial branching morphogenesis of embryonic mouse salivary gland, pancreas and kidney rudiments in culture, indicating that it may play a role as a morphogen during mammalian organogenesis. In developing pancreas and salivary gland rudiments, activin causes severe disruption of normal lobulation patterns of the epithelium whereas follistatin, an activin-binding protein, counteracts the effect of activin. In the kidney, activin delays branching of the ureter bud and reduces the number of secondary branches. TGF-[beta] induces a pattern of aberrant branching in the ureter bud derived epithelium distinct from that seen for activin. Reverse-transcriptase polymerase chain reaction, Northern hybridization and in situ hybridization analyses indicate that these developing tissues express the mRNA transcripts for activin subunits, follistatin or activin receptors. Our results are suggestive of a potential role for the activin-follistatin system as an intrinsic regulator of epithelial branching morphogenesis during mammalian organogenesis.


http://www.sciencedirect.com/science/article/B6T9H-3TVXPY2-2/2/eb15a9ff7e6d023a3ed50957deabe16d

We have examined the expression and function of the heterotrimeric GTP-binding protein Gq during early Xenopus embryogenesis. Abundant XG[alpha]q transcripts were detected in oocytes and early embryos by Northern blot analysis. In situ hybridization revealed that these transcripts are confined to the animal hemisphere of the mature oocyte and to the presumptive ectoderm of cleaving embryos. Microinjection at the two-cell stage of [alpha]q and Q209L[alpha]q,
constitutively activated mutant, causes a disruption in ectodermal cell adhesion at late gastrulation. Dissociation/reaggregation experiments performed on animal cap explants clearly demonstrate that the Q209L[alpha]q-induced phenotype occurs after reaggregation of the explants with a time-course similar to that observed in whole embryos. RT-PCR experiments performed on the explants from Q209L[alpha]q-injected embryos revealed a selective decrease in the amount of EP-cadherin mRNA. Co-injection of EP-cadherin RNA, but also E-cadherin RNA, rescued the disaggregated phenotype. These data emphasize the functional link between Gq protein-coupled signalling pathways and cadherin molecules in the ectodermal layer during the morphogenetic movements of gastrulation.


http://www.sciencedirect.com/science/article/B6T9H-41WC91G-2/2/cfb86c4d1a056d349d6affabb5b07c4b

Basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins form dimeric transcription factors to mediate diverse biological functions including xenobiotic metabolism, hypoxic response, circadian rhythm and central nervous system midline development. The Ah receptor nuclear translocator protein (ARNT) plays a central role as a common heterodimerization partner. Herein, we describe a novel, embryonically expressed, ARNT interacting protein (AINT) that may be a member of a larger coiled-coil PAS interacting protein family. The AINT C-terminus mediates interaction with the PAS domain of ARNT in yeast and interacts in vitro with ARNT and ARNT2 specifically. AINT localizes to the cytoplasm and overexpression leads to non-nuclear localization of ARNT. A dynamic pattern of AINT mRNA expression during embryogenesis and cerebellum ontogeny supports a role for AINT in development.


http://www.sciencedirect.com/science/article/B6T9H-4CNCT3Y-15/2/01aba50ed0f08aa1fdee8b4d47681b0c

Transformer-2 (Tra2), an RNA-binding protein, is an important regulator in Drosophila sex determination. In vertebrates, however, the role of Tra2 homologues is not known. We identified two teleost homologues of Tra2, which we named Tra2a and Tra2b, in medaka (Oryzias latipes). Furthermore, we demonstrated that both Tra2 mRNAs were predominantly expressed in germ cells of both sexes before the onset of sex differentiation, suggesting that both Tra2 homologues might be involved in the sex differentiation in medaka.


http://www.sciencedirect.com/science/article/B6T9H-3VYXXTM-1/2/de2653189cf412699c6dced32a9e7064

In an effort to isolate genes required for heart development and to further our understanding of
cardiac specification at the molecular level, we screened PlacZ enhancer trap lines for expression in the Drosophila heart. One of the lines generated in this screen, designated B2-2-15, was particularly interesting because of its early pattern of expression in cardiac precursor cells, which is dependent on the homeobox gene tinman, a key determinant of heart development in Drosophila. We isolated and characterized a gene in the vicinity of B2-2-15 that exhibits an identical expression pattern than the reporter gene of the enhancer trap. The product of this gene, apontic (apt; see also Gellon et al., 1997), does not appear to have any homology with known genes. apt mutant embryos show distinct abnormalities in heart morphology as early as mid-embryonic stages when the heat tube assembles, in that segments of heart cells (those of myocardial and pericardial identity) are often missing. Most strikingly, however, apt mutant embryos or larvae only develop a much reduced heart rate, perhaps because of defects in the assembly of an intact heart tube and/or because of defects in the function or physiological control of the myocardial cells, which normally mediate heart contractions. These cardiac defects may be the cause of death of these mutants during late embryonic or early larval stages.


http://www.sciencedirect.com/science/article/B6T9H-3W2TBCW-2/2/427b27f9a11de50f0b32786159a2

Syndecans are a family of heparan sulfate proteoglycans implicated in cell-cell and cell-matrix interactions. To investigate the roles of syndecans in early development, we identified three syndecan family members in Xenopus laevis: Xsyn-1, Xsyn-2, and Xsyn-3. Xsyn-1 and Xsyn-2 are maternal mRNAs localized to the animal pole in blastulae, and are expressed in the ectoderm of gastrulae. In neurulae, Xsyn-1 is restricted to non-neural ectoderm and Xsyn-2 is restricted to neural ectoderm. In tailbud embryos, the three syndecans are expressed in adjacent, non-overlapping patterns. Xsyn-2 is expressed in the heart while Xsyn-1 is expressed in the underlying anterior endoderm. Xsyn-3 is expressed in the hindbrain, midbrain, and forebrain, while Xsyn-2 is expressed in the intervening regions. These results suggest that different members of the syndecan family have distinct developmental roles, perhaps acting as barriers to define tissue boundaries.


http://www.sciencedirect.com/science/article/B6T9H-491V0TK-BS/2/4ce61c3c52d835e9c8f9c24f5c44d846

The Drosophila segment-polarity gene fused (fu) is required for pattern formation within embryonic segments and imaginal discs. We previously reported that the 5' part of the fused gene is homologous to the catalytic domain of serine/threonine kinases. We present here the sequence of the complete transcription unit, which predicts a 805 amino acid long protein. The kinase domain actually corresponds to 268 amino acids in the N-terminal part, and no known function can be attributed to the rest of the putative FUSED protein. Transcripts from the fused gene have been characterized: a unique 3.2 kb fused transcript is produced in nurse cells, in low abundance, from stage 8 of oogenesis, and persistently through the rest of oogenesis. In embryos, this transcript is evenly distributed in all embryonic cells until the extended germ band stage, after which its amount strongly decreases. Ubiquitous expression is detected later in imaginal wing and leg discs. Possible roles of the FUSED protein in signal transduction pathways required for intercellular communication at different stages of development are discussed.
Maternally synthesised factors contribute to the establishment of the germ cell lineage in lower vertebrates. In zebrafish, germ-soma segregation appears to be completed by the late blastula stage of development. To search for new germ cell factors in the zebrafish, we have used subtractive cDNA cloning. Here we report that linker histone H1M transcripts mark the germ line from the early gastrulation up to 18 h post-fertilisation.

**Metabolic Engineering** (2)


Random mutagenesis and directed evolution has been successfully used to improve desired properties of enzymes for biocatalysis and metabolic engineering. Here we employ the method to increase copy number of a pBBR-based broad host range plasmid, which can be used to express desired enzymes in a variety of microbial hosts. Localized random mutagenesis was performed in the replication control region of a pBBR-derived plasmid containing a [beta]-carotene reporter. Mutant plasmids were isolated that showed increased [beta]-carotene production. Real-time PCR analysis confirmed that the copy number of the mutant plasmids increased 3-7 fold. Sequence of the 10 mutant plasmids indicated that each plasmid contained single or multiple mutations in the rep gene or the flanking regions. Single amino acid change of serine to leucine at codon 100 of the replication protein and single nucleotide change of C to T at 46 bp upstream of the rep gene caused the increase of plasmid copy number. The utility of the mutant plasmids for metabolic engineering were further demonstrated by increased [beta]-carotene production, when an isoprenoid pathway gene (dxs) was co-expressed on a compatible plasmid. The mutant plasmids were tested in Agrobacterium tumefaciens. Increase of plasmid copy number and [beta]-carotene production was also observed in the non-Escherichia coli host.


Synthetic zinc finger transcription factors (ZFP-TFs) were designed to upregulate the expression of the endogenous Arabidopsis [gamma]-tocopherol methyltransferase (GMT) gene. This gene encodes the enzyme responsible for the conversion of [gamma]-tocopherol to [alpha]-tocopherol,
the tocopherol species with the highest vitamin E activity. Five three-finger zinc finger protein (ZFP) DNA binding domains were constructed and proven to bind tightly to 9 bp DNA sequences located in either the promoter or coding region of the GMT gene. When these ZFPs were fused to a nuclear localization signal and the maize C1 activation domain, four of the five resulting ZFP-TFs were able to upregulate the expression of the GMT gene in leaf protoplast transient assays. Seed-specific expression of these ZFP-TFs in transgenic Arabidopsis produced several lines with a heritable elevation in seed [alpha]-tocopherol. These results demonstrate that engineered ZFP-TFs comprised of plant-derived elements are capable of modulating the expression of endogenous genes in plants.

Metabolism (6)


http://www.sciencedirect.com/science/article/B6WN4-4D4T0WJ-R/2/730400e89fa7a2349546e4db4e90c1

We examined the effects of combined genotypes of the [beta]2-adrenergic receptor (AR) Arg16-Gly and [beta]3-AR Trp64-Arg polymorphisms on longitudinal serum total (T-C) and low-density lipoprotein cholesterol (LDL-C) profiles in 1,198 subjects examined multiple times (6,488 observations) from 1973 to 1996 in the Bogalusa Heart Study, at ages from 4.5 to 38 years. Within 5-year age groups, T-C was significantly (P < 0.05) higher in Arg16/Arg16 homozygotes than in Gly16 carriers among those 4 to 8 (171.4 +/- 30.0 v 161.5 +/- 27.7 mg/dL), 9 to 13 (167.7 +/- 28.6 v 162.4 +/- 27.4 mg/dL), and 14 to 18 (158.8 +/- 29.6 v 154.7 +/- 27.5 mg/dL) years of age, but not in those 19 to 23, 24 to 28, 29 to 33, or 34 to 38 years of age. The [beta]3-AR polymorphism was not associated with variation in either T-C or LDL-C. In multilevel polynomial growth curve models, the combination of the [beta]2-AR Arg16/Arg16 genotype with either the [beta]3-AR Arg64/Arg64 or Trp64/Arg64 genotypes, denoted AA/AX, was associated with variation in longitudinal T-C (P < 0.001) and lipid profiles differed among race/sex groups, being most marked in black females, in whom the AA/AX combination was associated with higher T-C and LDL-C profiles across all ages. In White males, the AA/AX combination was most strongly associated with higher lipids in adults. In black males and white females, lipid profiles differed little between genotype groups. Our findings suggest that the [beta]2-AR Arg16-Gly genotype influences T-C and LDL-C levels in an age-specific manner, that it may interact with [beta]3-AR Trp64-Arg genotypes to influence longitudinal T-C and LDL-C profiles, and that the effect of combined [beta]2/[beta]3-AR genotypes on T-C and LDL-C profiles may differ among race/sex groups.


http://www.sciencedirect.com/science/article/B6WN4-4D6XM8X-P/2/71e015d9814feeb4834df33c865f83e8
The influence of thyroid hormones on human adipose tissue leptin production and leptin gene expression was investigated in vitro and in vivo. Twelve women received 60 [μg] triiodothyronine (T3) per day for 7 days, which increased total T3 by 195% (1.78 +/- 0.07 to 5.25 +/- 0.39 mU/L, P < P < 0.05). Human subcutaneous adipose tissue biopsies from eight healthy women were incubated in vitro as small fragments with T3 in concentrations from 1 to 50 nmol/L. Leptin production was inhibited dose-dependently. After 24 hours of incubation, a T3 concentration of 50 nmol/L reduced basal leptin production by 42% (P < 0.05). T3 decreased basal leptin mRNA expression by 47% compared with controls (P < 0.05). High concentrations of T3 (>20 nmol/L) inhibited leptin production and leptin gene expression in vitro, whereas an elevation of T3 corresponding to a moderate thyrotoxic state (T3 5.25 +/- 0.39 nmol/L) was without any impact on serum leptin levels in vivo.


http://www.sciencedirect.com/science/article/B6WN4-4D6XKW1-BG/2/6ecac3d43fc9168afdcbac4302ae04c

Alternative splicing of the 36--base pair exon 11 of the human insulin receptor (IR) gene and of the corresponding domain of the rat IR gene results in the synthesis of two IR isoforms with distinct functional characteristics. Altered expression of these IR isoforms has been previously demonstrated in the skeletal muscle of patients with non--insulin-dependent diabetes mellitus (NIDDM); however, this observation was not confirmed by other studies and is still a matter of debate. To assess whether the reported altered isoform expression is due to the secondary metabolic derangement of diabetes, we examined alternative splicing of IR mRNAs (IR36+ and IR36-, corresponding to human Ex11+ and Ex11-) in the skeletal muscle and liver of 6-hour fasting 90% pancreatectomized insulin-resistant diabetic and control Sprague-Dawley rats, using the reverse transcriptase--polymerase chain reaction (PCR) technique. Both diabetic and control rats showed the same pattern of IR mRNA expression: the liver exclusively expressed IR36+ mRNA, whereas only IR36- mRNA was detected in muscle. In conclusion, diabetes mellitus per se does not alter the expression of IR isoforms in the liver and skeletal muscle, and therefore, at least in this animal model of NIDDM, impaired insulin action develops independently from a relative increase in IR36+ mRNA expression in skeletal muscle.


http://www.sciencedirect.com/science/article/B6WN4-490H4VJ-X/2/c8b9f4173d4cfe1cfcfd1461f5473b4ce

Mutations in adenosine triphosphate (ATP)-binding cassette transporter 1 (ABCA1) gene have been established as the molecular defect in Tangier disease and familial hypoalphalipoproteinemia, uncommon genetic disorders characterized by deficient or depressed high-density lipoprotein (HDL) cholesterol and increased triglycerides. However, information regarding the frequency of common variants, including Arg219Lys (R219K) within the coding region of the ABCA1 gene and their effect on these phenotypes in the general population is limited. This study examined the frequency and phenotypic effect of R219K variant in a community-based sample of 887 white and 390 black young adults aged 20 to 38 years. The frequency of the variant allele (K219) was higher in blacks than in whites (0.595 vs 0.262, P < 0.05). Carriers (KK+RK), unlike noncarriers (RR) showed a positive relationship between age and HDL cholesterol (regression coefficient [beta] = 0.28, P = 0.029 for
carriers $\beta = -0.18$, $P = .112$ for noncarriers). In addition, the variant allele attenuated the adverse positive relationship between BMI and triglycerides ($\beta = 0.032$, $P < .001$ for noncarriers). These results indicate that the K219 allele frequency differs markedly between blacks and whites, and that the variant-allele modulates the association between age and HDL cholesterol, as well as body fatness and triglycerides in a beneficial manner only in whites.


http://www.sciencedirect.com/science/article/B6WN4-4FHJ9TP-P/2/1d74028a8ffe955a62d3eb0d8835d4a9

Intercellular adhesion molecule-1 (ICAM-1) is involved in inflammation and development of atherosclerotic change of vascular endothelium. The aim of the present study is to investigate whether K469E polymorphism of the ICAM-1 gene is associated with various clinical factors including plasma fibrinogen in patients with type 2 diabetes. ICAM-1 gene polymorphism was examined using polymerase chain reaction and restriction enzyme analysis in 360 type 2 diabetic patients. Plasma fibrinogen levels and other clinical variables were measured as well as circulating soluble ICAM-1 (sICAM-1) levels by enzyme-linked immunosorbent assay. The distribution of ICAM-1 genotypes, EE, EK, and KK, was not significantly different between type 2 diabetes and 152 healthy control subjects. Among 3 groups according to ICAM-1 genotypes in type 2 diabetes, no difference was found in adiposity, glycemic control, lipid profile, insulin sensitivity evaluated by homeostasis model assessment, or sICAM-1. Regarding fibrinogen, the patients with E allele showed significantly lower plasma fibrinogen levels in a dose-dependent manner ($P = .033$). Spearman rank correlation analyses revealed that ICAM-1 genotype showed significant correlation with plasma fibrinogen level ($P R^2 = 0.148$, $P < .001$). In conclusion, K469E polymorphism of the ICAM-1 gene had impact on plasma fibrinogen level independently of other clinical factors in 360 type 2 diabetic patients, suggesting that fibrinogen is a candidate which links the ICAM-1 gene polymorphism to atherosclerosis.


http://www.sciencedirect.com/science/article/B6WN4-4D6XKP9-92/2/8d44da8220b4da43689ae4c75f51676

A combined $(GTT)_n (ATT)_n$ trinucleotide-repeat polymorphism designated as RAD1 has been identified at intron 2 of the rad gene on chromosome 16q. An association between the total length of the RAD1 locus and type 2 diabetes has been shown in white American subjects, but not in Finns. We genotyped 115 Japanese patients with type 2 diabetes and 114 nondiabetic control subjects at the RAD1 locus by the direct sequencing method, and found 16 RAD1 alleles composed of various combinations of GTTs and ATTs. Allele 14 consisting of four GTTs and seven ATTs accounted for the majority in both control subjects and diabetic patients, suggesting that RAD1 polymorphism is not a major genetic component for susceptibility to common forms of diabetes in the Japanese. There was no significant association between total repeat length and diabetes. However, the frequency of minor alleles containing five GTTs or three GTTs was significantly higher in diabetic patients versus nondiabetic subjects ($4.8\% v 0.9\%, P = .012$). Thus, genetic variability at the rad gene in linkage disequilibrium with RAD1 could be associated with a predisposition to type 2 diabetes in the Japanese population.

http://www.sciencedirect.com/science/article/B6WN5-45MGN48-8/2/54e4ae6759fa9a01acc5552df5daaae


http://www.sciencedirect.com/science/article/B6WN5-466CGKS-7/2/1a73cc2765a421ab59ca5065ccee8a845


http://www.sciencedirect.com/science/article/B6WN5-466CGKS-3/2/4ad918376908660b3bdf8695ec9f26


http://www.sciencedirect.com/science/article/B6WN5-46T3743-9/2/c0a7161677e1f3e5fccb1eae9ef15b6d3c


http://www.sciencedirect.com/science/article/B6WN5-49W60TF-B/2/2c5249679dfe760060dee9e9ead782ba50

Adapter-tagged competitive PCR (ATAC-PCR) is an advanced version of competitive quantitative PCR that is characterized by the addition of unique adapters to cDNA derived from each sample RNA. Using multiple adapters, we can accurately measure the relative expression ratios of many samples, with a calibration curve obtained from internal standards included in the same reaction. ATAC-PCR can identify differences in gene expression as small as twofold, even from very small amounts of sample RNA. This technique is suitable for confirming results obtained with cDNA microarrays or differential display, and it can process more than a thousand of genes per day when used in conjunction with a capillary DNA sequencer.
An increasing number of clinical cases of Hantavirus infections have been reported from various regions in Asia, Europe and North America. Hantaviruses (family Bunyaviridae, genus Hantavirus) are enveloped and possess a single-stranded trisegmented RNA genome of negative polarity. Rodents or insectivores are natural hosts of hantaviruses and transmit the virus to humans chiefly by aerosolisation. These viruses are the causative agents of haemorrhagic fever with renal and pulmonary syndromes. In the northeast of France, Puumala hantavirus causes, every year, more than 150 mild forms of haemorrhagic fever with a renal syndrome known as nephropathia epidemica. Serological tests may lack sensitivity for diagnosing early stages of infection and virus isolation is limited because it grows poorly in cell culture. Since reverse transcription (RT)-PCR amplification is an efficient method for detecting viral genomes in patient specimens, we developed an assay using a Taqman(R) probe and compared it with the classical RT-PCR amplification. To achieve this goal, a Puumala strain was grown in Vero E6 cells and RNA extracted from the culture supernatant. We found that the semi-nested RT-PCR detected a minimal amount of 300 TCID50 mL-1, while the Taqman(R) PCR allowed detection of less than 10 TCID50 mL-1 and provided a quantitative analysis.

Chlamydia trachomatis is an intracellular bacterium that causes ocular and urogenital diseases worldwide. Membrane proteins have only been partially characterized, and the discovery of a nine-member polymorphic membrane protein gene family has enhanced interest in defining their function. We previously reported two putative insertion sequence-like elements in pmpC for biovariant Ba and one each for G and L2, suggesting horizontal gene transfer. Because of this and the tissue tropism differences for these biovariants, we analyzed by quantitative real-time RT-PCR pmpC expression relative to immunogenic protein genes ompA, groEL and gseA throughout development. Sera from infected adolescents were reacted by immunoblot against recombinant (r)PmpC and rMOMP. ompA and groEL revealed different developmental transcriptome profiles among the biovariants. pmpC expression occurred at 2 h, peaked at 18 for L2 (at 24 for Ba and G), with the highest mRNA levels throughout development for L2. pmpC expression as a function of time paralleled ompA expression with higher mRNA levels compared with groEL later in development. Only sera from D-, E- and G-infected patients reacted to rPmpC; all infected patients reacted to rMOMP. pmpC expression during logarithmic growth suggests a role in membrane building and/or integrity, which is supported by the presence of a signal peptidase and C-terminal phenylalanine in PmpC. Because phylogenetic analyses of pmpC segregate serovars according to tissue tropism, we speculate that biovariant transcriptome differences may contribute to this tropism. The heterogeneous biovariant pmpC expression throughout development and differential PmpC immunoreactivity also suggest a role for pmpC in antigenic variation.

http://www.sciencedirect.com/science/article/B6VPN-491BJG-2/2/d78218441f2b529df3d08fbb27c99b40

Protective immunity to the parasite Trypanosoma cruzi in mice depends on a pro-inflammatory T cell response involving the production of interferon-[gamma] (IFN-[gamma]). In conjunction with interleukin-12 (IL-12), IL-18 promotes the synthesis of IFN-[gamma] and a T helper type 1 immune response. We investigated the requirements of IL-12 and IL-18 in murine T. cruzi infection by use of C57BL/6 mice genetically deficient in either cytokine. IL-12p40-/- mice succumbed to infection at doses of 100 parasites, whereas IL-18-/- and wild-type mice resisted infectious doses up to 1000 parasites to the same extent. Levels of parasitemia were comparable between the latter groups, as were tissue parasite burdens according to quantitative real-time PCR. In contrast, IL-12p40-/- mice displayed vastly increased levels of parasites both in blood and in tissue. IFN-[gamma] concentrations in the serum of infected mice and in supernatants of splenocytes stimulated in vitro were decreased in IL-18-/- mice, whereas in IL-12p40-/- mice, IFN-[gamma] was undetectable in the serum and drastically reduced in cell supernatants. Levels of IL-12 production were generally comparable between wild-type and IL-18-/- mice, as were levels of IL-4, IL-2 and nitric oxide. Thus, the requirement for endogenous pro-inflammatory cytokines for a protective murine immune response against T. cruzi is satisfied by the expression of IL-12, while IL-18 is dispensable.


http://www.sciencedirect.com/science/article/B6VPN-4CTJ467-1/2/2d0844cf04fb6a20cb3ebd85d4e0c812

Microsporidia are obligate intracellular parasites that cause opportunistic infections in AIDS and other immunocompromised patients. Eight simian immunodeficiency virus (SIV)-infected rhesus macaque monkeys (Macaca mulatta) were inoculated orally with Enterocytozoon bieneusi spores isolated from intestinal lavage fluid of an AIDS patient (genotype D) to study the natural history of this infection. Four monkeys were already naturally infected with E. bieneusi (also genotype D), and were included to determine if a second inoculum affected the course of illness. Spore shedding was detected in feces of all eight monkeys within the first week of experimental infection. Five monkeys died within 3.5 months of experimental E. bieneusi inoculation. Three of these five monkeys began the study with CD4+CD29+ T cell levels well below 20% of total T lymphocytes. Deaths were due to a variety of AIDS-related manifestations. Microsporidia did not appear to directly contribute to mortality but may have contributed to morbidity. At necropsy, microsporidia were found in bile and tissue sections of the gallbladder but not in the gut, kidneys, or liver. The percent CD4+CD29+ levels of the last three monkeys remained near the level observed at the time of inoculation. These monkeys lived more than 2 years after the end of the study and continued to shed spores. This study corroborates previous reports that E. bieneusi can be reliably transmitted to SIV-infected rhesus monkeys but indicates that the use of SIV-infected monkeys for the study of microsporidiosis is complicated by the confounding effect of other opportunistic or AIDS-related infections.

http://www.sciencedirect.com/science/article/B6VPN-47RB11M-1/2/a6d7f3bd262e202e611386154a1e89da

A major problem of infections with facultative intracellular bacteria is their chronic course. We comprehensively evaluated the host response in murine brucellosis to study mechanisms contributing to bacterial persistence in the presence of an established immune response. Evidence is presented that the decrease in eradication kinetics, reproducibly occurring 18 d after infection of mice with Brucella abortus S19, is related to a state of downregulation of defense mechanisms. This is not due to a Th1 to Th2 switch or prostaglandin-mediated suppression by macrophages but is most probably caused by a severe disruption of spleen morphology at the height of Brucella-induced delayed type hypersensitivity. This results in a profound depletion of both CD4+ and CD8+ T cells in periarteriolar lymphatic sheaths, a consecutive deleterious shift in the relation of permissive macrophages and protective lymphocytes and an impaired capacity of splenocytes to produce IFN-[gamma] in response to soluble Brucella antigen.


http://www.sciencedirect.com/science/article/B6VPN-4BSWK35-2/2/199beb7f1c444a84aa673ae0615d2474d

Interferon (IFN)-[gamma] plays an essential role in host defense against infection with Mycobacterium tuberculosis, and its synthesis is critically regulated by interleukin (IL)-12, IL-18 and the recently identified IL-23. The present study was designed to determine the roles of these cytokines in IFN-[gamma]-mediated host defenses against M. tuberculosis. For this purpose, we compared host protective responses in IL-12p40 and IL-18 double-knockout (DKO) mice (which lacked both IL-12/IL-18 and also IL-23) and IFN-[gamma] gene-disrupted (GKO) mice. DKO mice were more resistant to the infection than GKO mice, as indicated by their extended survival and reduced live colony numbers in spleen, liver and lung. IFN-[gamma] was detected by ELISA in liver and lung homogenates, but not in spleen and serum, and in all organs by RT-PCR in DKO mice at comparable or reduced levels to those in wild-type mice. IFN-[gamma] production was reduced by depletion of CD4+ T cells, but not of natural killer (NK), NKT, [gamma][delta]T and dendritic cells. Neutralization of IFN-[gamma] or TNF-[alpha] by specific monoclonal antibodies (mAbs) significantly shortened the survival time of the infected DKO mice. Furthermore, anti-TNF-[alpha] mAb partially attenuated IFN-[gamma] synthesis in the liver of these mice. Finally, the expression level of inducible nitric oxide synthase (iNOS) mRNA in the spleen, liver and lung was considerable in DKO mice but only marginal or undetected in GKO mice. Our results indicate the presence of IL-12-, IL-18- and IL-23-independent host protective responses against mycobacterial infection mediated by IFN-[gamma], which was secreted from helper T cells.


http://www.sciencedirect.com/science/article/B6VPN-490R86X-
Amplified fragment length polymorphism (AFLP) was applied to 35 and 34 isolates, respectively, of Salmonella enterica serovar Typhimurium phage types DT 9 and DT 135, using eight primer pair combinations. Eight and 17 AFLP types were observed in DT 9 and DT 135, respectively. DT 9 is rare in the UK and common in Australia, but one AFLP form dominated with 28 isolates, comprising 22 of 25 UK isolates, four of five Australian isolates, one Jamaican and one Spanish isolate. Of the others, two UK isolates are closely related to the major form, two from elsewhere are in the major cluster and three isolates from different countries are in a separate cluster. For DT 135, two closely related AFLP types of seven and 11 isolates form the major cluster, which also includes 11 isolates, mostly in single-isolate AFLP types, while five isolates from different countries form a well-separated minor cluster. For both DTs all isolates are grouped together if only the phage type specific bands identified earlier are used, confirming their value for molecular-based 'phage typing'. Polymorphic markers identified in this study could also be used for subtyping within both phage types. The value of AFLP is in locating DNA fragments useful for typing, but implementation of a replacement typing scheme would probably involve multiplex PCR or microarray technologies.


Borna disease virus (BDV) is a non-cytolytic, neurotropic RNA virus that has a broad host range in warm-blooded animals, probably including humans. Recently, we have demonstrated that the neonatal gerbil is a unique model for analyzing BDV-induced acute neurological disease. In this report, to understand the effects of the brain development of gerbils in BDV-induced neuropathogenesis, as well as to investigate the host-dependent differences in BDV propagation and pathogenesis in the brains, we performed experimental infection of BDV using two different infant rodent models, gerbils and rats. We demonstrated here that most of the gerbils infected with BDV on postnatal days (PD) 14, but not on PD1 and PD7, could survive neurological disorders during the observation period of PD85. Interestingly, the levels of BDV RNA and antigen in surviving PD14 inoculated gerbil brains were extremely low, whereas diseased gerbils and both PD7 and PD14 inoculated rats contained significant amounts of BDV antigen in the central nervous system, suggesting that PD14 gerbils successfully controlled BDV spread in the brain. Furthermore, the viral distribution, as well as the expression levels of cytokine and CD8 mRNAs, in the brains was markedly different between the rodent models and between diseased and non-diseased statuses of the gerbils. These results demonstrated that developmentally regulated and host-specific factors could contribute to the prevention of BDV spread in developing animal brains. Studies using different animal systems would provide novel insights into the mechanisms of host defense responses to neurotropic virus infections.


The transmissibility of the GB virus C (hepatitis G virus; HGV), a member of the Flaviviridae, by a typical flavivirus vector was investigated. Female mosquitoes of the species Aedes aegypti were
fed with HGV-infected human blood and assayed 1, 24, 48, 72 and 96 h after the blood meal for viral RNA, human glyceraldehyde-3-phosphate dehydrogenase mRNA, human [beta]-actin DNA and A. aegypti actin mRNA by total nucleic acid extraction, reverse transcription and PCR. Viral RNA had already disappeared from nucleic acid extracts 1 h after the blood meal and was not detectable throughout the observation period. Aedes-specific mRNA served as an internal control and was detected in all nucleic acid extracts, whereas human mRNA had disappeared after 24 h, indicating digestion of human cells. From these results we conclude that GB virus C (HGV) cannot replicate in A. aegypti, which is a widespread and competent vector of several other flaviviruses.

Microbial Pathogenesis (2)


http://www.sciencedirect.com/science/article/B6WN6-4B4HB5D-1/2/beb5fa049ba30acb919949315dfc9bb4

Molecular-genetic properties of classical biotype Vibrio cholerae strains that caused the Asiatic cholera outbreak in 1942 in Russia have been investigated for the first time. Being characterized by high-level production of cholera toxin and toxin-coregulated adhesion pili both of which are the major virulence factors, all the strains studied, in contrast to the typical cholera pathogens, were autographic requiring purine and/or amino acids added to the minimal medium for their growth. Moreover, these strains containing the structural gene hapA, as shown by the polymerase chain reaction, produced no soluble hemagglutinin/protease, which enables the vibrios to get disseminated in the environment. The peculiarities of the natural V. cholerae strains elucidated in the work are likely to be responsible for the unusual infectious and epidemic processes observed during that cholera outbreak.


http://www.sciencedirect.com/science/article/B6WN6-4DBSV92-1/2/8a961e1228e023d6ff446280eeb1f501

We investigated mechanisms involved in killing of mycobacterial organisms by comparing the response of bovine monocyte-derived macrophages to ingestion of Mycobacterium avium subsp. paratuberculosis or M. avium subsp. avium organisms. Previous studies have shown that bovine macrophages have the capacity to kill M. avium subsp. avium organisms in vitro but cannot kill M. avium subsp. paratuberculosis organisms. We used bovine cDNA microarray technology to investigate sequential gene expression by bovine monocyte-derived macrophages and function assays to correlate gene expression with biological activity. Results of the gene expression studies indicated substantial differences between macrophages phagocytizing the two organisms. At 2, 6, and 24 h after infection, 12, 53, and 19 genes, respectively, were differentially expressed. Over all time periods, approximately twice as many genes had lower expression in M. avium
subsp. paratuberculosis-infected macrophages than had greater expression. Differentially regulated genes of most interest to antimicrobial responses included inflammatory molecules (transforming growth factor-[beta], thrombospondin 1, monocyte chemokine, and cathepsin K), phagosome-lysosome-related genes (H+ ATPases, lysosomal-associated membrane protein 2, vesicle trafficking protein, and solute carrier protein), and apoptosis-related genes (tumor necrosis factor receptor-associated factor 2, and tumor protein p53 binding protein). Function assays indicated that M. avium subsp. avium-infected macrophages had a greater capacity to acidify phagosomes and a greater percentage of apoptotic cells. In conclusion, these results suggest that a complex interaction between macrophages and mycobacterial organisms is involved in determining the fate of the organism. Although multiple genes and metabolic pathways are involved, the capacity of cells to acidify phagosomes and induce apoptosis appears to play a prominent role.

Microbiological Research (1)


Summary
The study examined the diversity of ectomycorrhizal fungi, naturally established on roots of containerised Pinus seedlings in a nursery, using PCR-RFLP and sequencing of the nuclear ribosomal internal transcribed spacer. Seventy-two samples, including ectomycorrhizae and fruit bodies, were examined. Molecular typing assigned the fungal symbionts to four ectomycorrhizal Boletales: Rhizopogon rubescens, Suillus bovinus, S. variegatus, and R. luteolus. R. rubescens was abundant (37.5%), while Suillus and R. luteolus species were moderately established (25-26%) and rare (2.8%), respectively. In addition, Rhizopogon species colonised P. nigra ssp. salzmannii seedlings, whereas Suillus species were identified on Pinus nigra ssp. nigra seedlings. The diversity and the ability of these naturally established symbionts under artificial nursery conditions were discussed. The molecular survey investigated here should contribute to successful monitoring of mycorrhizal application under both nursery and plantation conditions.

Microbiology (22)


http://mic.sgmjournals.org/cgi/content/abstract/151/3/789

Nematode-trapping fungi enter the parasitic stage by developing specific morphological structures
called traps. The global patterns of gene expression in traps and mycelium of the fungus Monacrosporium haptotylum were compared. The trap of this fungus is a unicellular spherical structure called the knob, which develops on the apex of a hyphal branch. RNA was isolated from knobs and mycelium and hybridized to a cDNA array containing probes of 2822 EST clones of M. haptotylum. Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. In total, 23.3% (657 of 2822) of the putative genes were differentially expressed in knobs versus mycelium. Several of these genes displayed sequence similarities to genes known to be involved in regulating morphogenesis and cell polarity in fungi. Among them were several putative homologues for small GTPases, such as rho1, rac1 and ras1, and a rho GDP dissociation inhibitor (rdi1). Several homologues to genes involved in stress response, protein synthesis and protein degradation, transcription, and carbon metabolism were also differentially expressed. In the last category, a glycogen phosphorylase (gph1) gene homologue, one of the most upregulated genes in the knobs as compared to mycelium, was characterized. A number of the genes that were differentially expressed in trap cells are also known to be regulated during the development of infection structures in plant-pathogenic fungi. Among them, a gas1 (mas3) gene homologue (designated gks1), which is specifically expressed in appressoria of the rice blast fungus, was characterized.


http://mic.sgmjournals.org/cgi/content/abstract/149/7/1687

Bacillus sphaericus, a bacterium of biotechnological interest due to its ability to produce mosquitoicidal toxins, is unable to use sugars as carbon source. However, ptsHI genes encoding HPr and EI proteins belonging to a PTS were cloned, sequenced and characterized. Both HPr and EI proteins were fully functional for phosphoenolpyruvate-dependent transphosphorylation in complementation assays using extracts from Staphylococcus aureus mutants for one of these proteins. HPr(His6) was purified from wild-type and a Ser46/Gln mutant of B. sphaericus, and used for in vitro phosphorylation experiments using extracts from either B. sphaericus or Bacillus subtilis as kinase source. The results showed that both phosphorylated forms, P-Ser46-HPr and P-His15-HPr, could be obtained. The findings also proved indirectly the existence of an HPr kinase activity in B. sphaericus. The genetic structure of these ptsHI genes has some unusual features, as they are co-transcribed with genes encoding metabollic enzymes related to N-acetylglucosamine (GlcNAc) catabolism (nagA, nagB and an undetermined orf2). In fact, this bacterium was able to utilize this amino sugar as carbon and energy source, but a ptsH null mutant had lost this characteristic. Investigation of GlcNAc uptake and streptozotocin inhibition in both a wild-type and a ptsH null mutant strain led to the proposal that GlcNAc is transported and phosphorylated by an EIINag element of the PTS, as yet uncharacterized. In addition, GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase activities were determined; both were induced in the presence of GlcNAc. These results, together with the authors' recent findings of the presence of a phosphofructokinase activity, are strongly indicative of a glycolytic pathway in B. sphaericus. They also open new possibilities for genetic improvements in industrial applications.


http://mic.sgmjournals.org/cgi/content/abstract/148/1/297
A nitroreductase with distinct properties that can activate the prodrug 5-aziridinyl-2,4-dinitrobenzamide (CB 1954) was isolated from Bacillus amyoliquefaciens. The encoding gene was identified as a homologue of the ywrO of Bacillus subtilis, and was obtained as a PCR product by reverse genetics, cloned and the entire nucleotide sequence determined. The gene was found to reside between homologues of the B. subtilis alsD and yswB genes; however, the ywrO and yswB genes of B. amyoliquefaciens were not separated by a fourth gene, ywsA. The B. amyoliquefaciens ywrO gene was overexpressed, the recombinant protein purified and its properties were compared with those of two CB 1954-activating enzymes, Escherichia coli B nitroreductase (NTR) and Walker DT-diaphorase (DTD). In common with these enzymes menadione was an electron acceptor (Km 3 \(\mu\)M) and activity with this substrate was inhibited by the presence of dicoumarol (Ki 1\(\cdot\)0 \(\mu\)M). In contrast, YwrO showed a marked preference for NADPH as a cofactor (Km 40 \(\mu\)M) and therefore could not be classified as a DTD (EC 1.6.99.2). The flavin FMN was an acceptor with high affinity. B. amyoliquefaciens YwrO was shown to be a flavoprotein with a monomeric molecular mass of 21\(\cdot\)5 kDa by calculation and SDS-PAGE. The cytotoxic 4-hydroxylamine derivative was the single CB 1954 reduction product, but B. amyoliquefaciens YwrO was inactive with the bischloroethyl analogue of CB 1954, SN 23862. In both of these properties B. amyoliquefaciens YwrO more closely resembles DTD than NTR. Its Km for CB 1954 was lower than that of NTR (617 \(\mu\)M compared to 862 \(\mu\)M). Enhanced in vitro cytotoxicity of CB 1954 was demonstrated on incubation of V79 cells with prodrug, NADPH and B. amyoliquefaciens YwrO. The work has led to the identification of a previously unknown nitroreductase, B. amyoliquefaciens YwrO, with distinct properties which will aid the rational selection of appropriate genes for applications in directed enzyme prodrug therapy (DEPT).


http://mic.sgmjournals.org/cgi/content/abstract/148/10/3007

The authors have developed a simple and highly efficient system for generating allelic exchanges in both fast- and slow-growing mycobacteria. In this procedure a gene of interest, disrupted by a selectable marker, is cloned into a conditionally replicating (temperature-sensitive) shuttle phasmid to generate a specialized transducing mycobacteriophage. The temperature-sensitive mutations in the mycobacteriophage genome permit replication at the permissive temperature of 30 \(\text{degrees}\)C but prevent replication at the non-permissive temperature of 37 \(\text{degrees}\)C. Transduction at a non-permissive temperature results in highly efficient delivery of the recombination substrate to virtually all cells in the recipient population. The deletion mutations in the targeted genes are marked with antibiotic-resistance genes that are flanked by \(\text{gamma}\}\{\text{delta}\}-\text{res} (\text{resolvase recognition target}) sites. The transductants which have undergone a homologous recombination event can be conveniently selected on antibiotic-containing media. To demonstrate the utility of this genetic system seven different targeted gene disruptions were generated in three substrains of Mycobacterium bovis BCG, three strains of Mycobacterium tuberculosis, and Mycobacterium smegmatis. Mutants in the lysA, nadBC, panC, panCD, leuCD, Rv3291c and Rv0867c genes or operons were isolated as antibiotic-resistant (and in some cases auxotrophic) transductants. Using a plasmid encoding the \(\text{gamma}\}\{\text{delta}\}-\text{resolvase} (\text{tnpR}), the resistance genes could be removed, generating unmarked deletion mutations. It is concluded from the high frequency of allelic exchange events observed in this study that specialized transduction is a very efficient technique for genetic manipulation of mycobacteria and is a method of choice for constructing isogenic strains of M. tuberculosis, BCG or M. smegmatis which differ by defined mutations.

http://mic.sgmjournals.org/cgi/content/abstract/151/1/59

Transcripts of the gas vesicle genes gvpA and gvpC were detected in single filaments of the cyanobacterium Planktothrix rubescens using reverse transcription and quantitative real-time PCR. Primers were designed to amplify short sequences within gvpA and three length variants of gvpC. With genomic template DNA, and using Sybr Green to monitor product accumulation, similar amplification efficiencies were observed for each of these genes. The relative copy numbers of gvpC length variants in genomic DNA from five Planktothrix gas vesicle genotypes determined by real-time PCR were similar to those indicated by sequencing the gas vesicle gene clusters. The precipitation of gvp cDNA reverse-transcribed from cellular RNA from single filaments was required before amplification of the gene fragments; without this step it was not possible to detect the accumulation of the expected amplicons by dissociation analysis. Precipitation was also necessary to ensure the generation of product curves that allowed linear regression in an early stage of PCR, a prerequisite for the quantification of low-input cDNA amounts without the need for standard curves. This report shows that different gvpC length variants are transcribed within single Planktothrix filaments, both from laboratory cultures and from natural samples taken from Lake Zurich. This has implications for the efficiency of buoyancy provision by the possible production of gas vesicles of different strengths within individual cyanobacterial filaments. The hypothesis that post-transcriptional regulation may influence the type of protein (GvpC) present in gas vesicles is presented.


http://mic.sgmjournals.org/cgi/content/abstract/151/3/941

The sequence of 50 625 bp of chromosomal DNA derived from Shiga-toxin (Stx)-producing Escherichia coli (STEC) O111: H- strain 1639/77 was determined. This DNA fragment contains the cryptic Stx1-encoding prophage CP-1639 and its flanking chromosomal regions. The genome of CP-1639 basically resembles that of lambdoid phages in structure, but contains three IS629 elements, one of which disrupts the gene of a tail fibre component. The prophage genome lacks parts of the recombination region including integrase and excisionase genes. Moreover, a capsid protein gene is absent. CP-1639 is closely associated with an integrase gene of an ancient integrative element. This element consists of three ORFs of unknown origin and a truncated integrase gene homologous to intA of CP4-57. By PCR analysis and sequencing, it was shown that this integrative element is present in a number of non-O157 STEC serotypes and in non-STEC strains, where it is located at the 3'-end of the chromosomal ssrA gene. Whereas in most E. coli O111: H- strains, prophages are inserted in this site, E. coli O26 strains contain the integrative element not connected to a prophage. In E. coli O103 strains, the genetic structure of this region is variable. Comparison of DNA sequences of this particular site in E. coli O157: H7 strain EDL933, E. coli O111: H- strain 1639/77 and E. coli K-12 strain MG1655 showed that the ssrA gene is associated in all cases with the presence of foreign DNA. The results of this study have shown that the cryptic prophage CP-1639 is associated with an integrative element at a particular site in the E. coli chromosome that possesses high genetic variability.

Three genes from the aminoethoxyvinylglycine (AVG)-producing Streptomyces sp. NRRL 5331 involved in threonine biosynthesis, hom, thrB and thrC, encoding homoserine dehydrogenase (HDH), homoserine kinase (HK) and threonine synthase (TS), respectively, have been cloned and sequenced. The hom and thrC genes appear to be organized in a bicistronic operon as deduced by disruption experiments. The thrB gene, however, is transcribed as a monocistronic transcript. The encoded proteins are quite similar to the HDH, HK and TS proteins from other bacterial species. The overall organization of these three genes, in the order hom-thrC-thrB, differs from that in other bacteria and is similar to that reported in the Streptomyces coelicolor genome sequence. This is the first time in which the gene cluster for the three last steps of threonine biosynthesis has been characterized from a streptomycete. Disruption of thrC indicated that threonine is not a direct precursor for AVG biosynthesis in Streptomyces sp. NRRL 5331 and suggested that the branching point of the aspartic acid-derived biosynthetic route of this metabolite should lie earlier on the threonine biosynthetic route.


Fifty strains belonging to Vibrio harveyi, Vibrio campbellii, and the recently described Vibrio rotiferianus, were analysed using phenotypic and genomic techniques with the aim of analysing the usefulness of the different techniques for the identification of V. harveyi-related species. The species V. harveyi and V. campbellii were phenotypically indistinguishable by more than 100 phenotypic features. Thirty-nine experimental strains were phenotypically identified as V. harveyi, but FAFLP, REP-PCR, IGS-PCR and DNA-DNA hybridization proved that they in fact belong to the species V. campbellii. Similar groupings were found among all fingerprinting methodologies (except IGS-PCR). Thirty-two experimental strains clustered with the V. campbellii type and one reference strain; seven strains clustered with the V. harveyi type and three reference strains; and the type and four reference strains of V. rotiferianus grouped together. The correlations between DNA-DNA hybridization and the genomic fingerprinting by FAFLP and (GTG)5-PCR were found to be above 0.68 and statistically significant, suggesting the value of the latter techniques for the reliable identification of V. harveyi-related species. The results presented indicate that strains phenotypically identified as V. harveyi are in fact V. campbellii; these findings position V. campbellii as an important species involved in diseases of reared aquatic organisms.


Variable-number tandem repeats (VNTRs) have been shown to be a powerful tool in the determination of evolutionary relationships and population genetics of bacteria. The sequencing of a number of Staphylococcus aureus genomes has allowed the identification of novel VNTR sequences in S. aureus, which are similar to those used in the study of the evolution of Mycobacterium tuberculosis clades. Seven VNTRs, termed staphylococcal interspersed repeat units (SIRUs), distributed around the genome are described, occurring in both unique and multiple sites, and varying in length from 48 to 159 bp. Variations in copy numbers were observed in all loci, within both the sequenced genomes and the UK epidemic methicillin-resistant S.
S. aureus (EMRSA) isolates. Clonally related UK EMRSA isolates were clustered using SIRUs, which provided a greater degree of discrimination than multi-locus sequence typing, indicating that VNTRs may be a more appropriate evolutionary marker for studying transmission events and the geographical spread of S. aureus clades.


http://mic.sgmjournals.org/cgi/content/abstract/148/7/2171

The gene encoding a haemagglutinin of H. paragallinarum, hagA, has been identified and the full-length nucleotide sequence determined. A [~]39 kDa protein, recognized by an anti-haemagglutinin monoclonal antibody, mAb4D, was purified from H. paragallinarum strain 0083 and the N-terminal sequence obtained. The full-length nucleotide sequence was obtained by inverse PCR and the deduced amino acid sequence of the protein encoded was shown to be similar to other outer-membrane proteins of closely related organisms in the HAP group (Haemophilus, Actinobacillus, Pasteurella), especially the P5 protein of Haemophilus influenzae. The hagA gene was cloned into a His-tag expression vector and overexpressed in Escherichia coli strain M15(pREP4). The identity of the purified recombinant protein as a H. paragallinarum haemagglutinin was confirmed by haemagglutination of chicken red blood cells and reactivity, in a Western blot, with the monoclonal antibody specific for the serovar A haemagglutinin.


http://mic.sgmjournals.org/cgi/content/abstract/149/7/1633

Medermycin is a Streptomyces aromatic C-glycoside antibiotic classified in the benzoisochromanequinones (BIQs), which presents several interesting biosynthetic problems concerning polyketide synthase (PKS), post-PKS tailoring and deoxysugar pathways. The biosynthetic gene cluster for medermycin (the med cluster) was cloned from Streptomyces sp. AM-7161. Completeness of the clone was proved by the heterologous expression of a cosm id carrying the entire med cluster in Streptomyces coelicolor CH999 to produce medermycin. The DNA sequence of the cosmid (36 202 bp) revealed 34 complete ORFs, with an incomplete ORF at either end. Functional assignment of the deduced products was made for PKS and biosynthetically related enzymes, tailoring steps including stereochemical control, oxidation, angolosamine pathway, C-glycosylation, and regulation. The med cluster was estimated to be about 30 kb long, covering 29 ORFs. An unusual characteristic of the cluster is the disconnected organization of the minimal PKS genes: med-ORF23 encoding the acyl carrier protein is 20 kb apart from med-ORF1 and med-ORF2 for the two ketosynthase components. Secondly, the six genes (med-ORF14, 15, 16, 17, 18 and 20) for the biosynthesis of the deoxysugar, angolosamine, are all contiguous. Finally, the finding of a glycosyltransferase gene, med-ORF8, suggests a possible involvement of conventional C-glycosylation in medermycin biosynthesis. Comparison among the three complete BIQ gene clusters - med and those for actinorhodin (act) and granaticin (gra) - revealed some common genes whose deduced functions are unavailable from database searches (the unknowns'). An example is med-ORF5, a homologue of actVI-ORF3 and gra-ORF18, which was highlighted by a recent proteomic analysis of S. coelicolor A3(2).

http://mic.sgmjournals.org/cgi/content/abstract/150/9/2857

Proteus mirabilis, a Gram-negative urinary tract pathogen, has two highly homologous, tandemly arranged flagellin-encoding genes, flaA and flaB. flaA is transcribed from a{sigma}28 promoter, while flaB is a silent allele. Previous studies have demonstrated the presence of a family of hybrid flagellin genes, referred to as flaAB. These genes are composed of the 5' end of flaA and the 3' end of flaB, and are produced through excision of the intervening DNA between the two genes. Although the existence of flaAB DNA has been documented, it was not known if transcription of flaAB occurs in wild-type P. mirabilis. In this study, proof of flaAB transcription was obtained from a combination of RNA dot-bLOTS and RT-PCR assays using specific primers and probes for flaAB and flaA. The RNA data were further supported by the demonstration of phenotypic switching of the locus using a FlaAB-detector strain. The results show that flaAB mRNA is transcribed and is 1/64 as abundant as flaA in the population of wild-type cells, suggesting that flaAB constitutes 1{middle dot}0-1{middle dot}5 % of the total flagellin message. Nucleotide sequence analysis of flaAB products produced by RT-PCR from the wild-type confirms previous reports of a variable fusion site between flaA and flaB resulting in a hybrid flagellin transcript. These data support the hypothesis that the production of FlaAB is integral to the physiology of P. mirabilis.


http://mic.sgmjournals.org/cgi/content/abstract/150/12/3947

The Yersinia pseudotuberculosis chromosome contains a seven-gene polycistronic unit (the pmrF operon) whose products share extensive homologies with their pmrF counterparts in Salmonella enterica serovar Typhimurium (S. typhimurium), another Gram-negative bacterial enteropathogen. This gene cluster is essential for addition of 4-aminoarabinose to the lipid moiety of LPS, as demonstrated by MALDI-TOF mass spectrometry of lipid A from both wild-type and pmrF-mutated strains. As in S. typhimurium, 4-aminoarabinose substitution of lipid A contributes to in vitro resistance of Y. pseudotuberculosis to the antimicrobial peptide polymyxin B. Whereas pmrF expression in S. typhimurium is mediated by both the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems, it appears to be PmrA-PmrB-independent in Y. pseudotuberculosis, with the response regulator PhoP interacting directly with the pmrF operon promoter region. This result reveals that the ubiquitous PmrA-PmrB regulatory system controls different regulons in distinct bacterial species. In addition, pmrF inactivation in Y. pseudotuberculosis has no effect on bacterial virulence in the mouse, again in contrast to the situation in S. typhimurium. The marked differences in pmrF operon regulation in these two phylogenetically close bacterial species may be related to their dissimilar lifestyles.


http://mic.sgmjournals.org/cgi/content/abstract/151/1/145

The nature of secreted aminopeptidases in Trichophyton rubrum was investigated by using a reverse genetic approach. T. rubrum genomic and cDNA libraries were screened with Aspergillus spp. and Saccharomyces cerevisiae aminopeptidase genes as the probes. Two leucine
aminopeptidases, ruLap1 and ruLap2, and two dipeptidyl-peptidases, ruDppIV and ruDppV, were characterized and compared to orthologues secreted by Aspergillus fumigatus using a recombinant protein from Pichia pastoris. RuLap1 is a 33 kDa nonglycosylated protein, while ruLap2 is a 58-65 kDa glycoprotein. The hydrolytic activity of ruLap1, ruLap2 and A. fumigatus orthologues showed various preferences for different aminoacyl-7-amido-4-methylcoumarin substrates, and various sensitivities to inhibitors and cations. ruDppIV and ruDppV showed similar activities to A. fumigatus orthologues. In addition to endopeptidases, the four aminopeptidases ruLap1, ruLap2, ruDppIV and ruDppV were produced by T. rubrum in a medium containing keratin as the sole nitrogen source. Synergism between endo- and exopeptidases is likely to be essential for dermatophyte virulence, since these fungi grow only in keratinized tissues.


http://mic.sgmjournals.org/cgi/content/abstract/148/12/3801

Bacterial accommodation to moderate concentrations of cadmium is accompanied by transient activation of general stress proteins as well as a sustained induction of other proteins of hitherto unknown functions. One of the latter proteins was previously identified as the product of the Escherichia coli yodA ORF. The yodA ORF encodes 216 aa residues (the YodA protein) and the increased synthesis of YodA during cadmium stress was found probably to be a result of transcriptional activation from one single promoter upstream of the structural yodA gene. Analysis of a transcriptional gene fusion, PyodA-lacZ, demonstrated that basal expression of yodA is low during exponential growth and expression is increased greater than 50-fold by addition of cadmium to growing cells. However, challenging cells with additional metals such as zinc, copper, cobalt and nickel did not increase the level of yodA expression. In addition, hydrogen peroxide also increased yodA expression whereas the superoxide-generating agent paraquat failed to do so. Surprisingly, cadmium-induced transcription of yodA is dependent on soxS and fur, but independent of oxyR. Moreover, a double relA spoT mutation abolished induction of yodA during cadmium exposure but ppGpp is not sufficient to induce yodA since expression of the gene is not elevated during stationary phase. After 45 min of cadmium exposure the YodA protein was primarily detected in the cytoplasmic fraction but was later (150 min) found in both the cytoplasmic and periplasmic compartments.


http://mic.sgmjournals.org/cgi/content/abstract/148/6/1871

This study was aimed at characterizing a cell-surface 25 kDa glycoprotein (GP25) that was previously shown to be underproduced by a spontaneous adhesion-defective mutant D5 of Ruminococcus albus 20. An antiserum against wild-type strain 20 was adsorbed with the mutant D5 to enrich it in antibodies specific to adhesion structures of R. albus 20. The resulting antiserum, called anti-Adh serum, blocked adhesion of R. albus 20 and reacted mainly with GP25 in bacterial and extracellular protein fractions of R. albus 20. The N-terminal sequence of purified GP25 was identical to that of CbpC, a 21 kDa cellulose-binding protein (CBP) of R. albus 8. The nucleotide sequence of the gp25 gene was determined by PCR and genomic walking procedures. The gp25 gene encoded a protein of 165 aa with a calculated molecular mass of 16940 Da that showed 72% identity with CbpC and presented homologies with type IV pilins of Gram-negative pathogenic bacteria. Negative-staining electron microscopy revealed fine and flexible pili surrounding R. albus 20 cells while mutant cells were not piliated. In addition,
immunoelectron microscopy showed that the anti-Adh serum probing mainly GP25, completely
decorated the pili surrounding R. albus 20, thereby showing that GP25 was a major pilus subunit.
This study shows for the first time the presence of pili at the surface of R. albus and identifies
GP25 as their major protein subunit. Though GP25 was not identified as a CBP, isolated pili were
shown to bind cellulose. In conclusion, these pili, which belong to the family of type IV pili,
mediate adhesion of R. albus 20 to cellulose.

Haemophilus paragallinarum.” Microbiology 149(11): 3177-3184.
http://mic.sgmjournals.org/cgi/content/abstract/149/11/3177

The full sequence of plasmid p250, isolated from Haemophilus paragallinarum strain HP250, has
been obtained. The plasmid contains seven ORFs: a putative integrase, a putative replication
protein (repB) and five ORFs similar to those from the haemocin (bacteriocin) hmcDCBAI operon
from Haemophilus influenzae. Of 19 other non-plasmid-containing H. paragallinarum strains
screened (11 serovar reference strains and 8 field isolates), 17 strains produced haemocin and
were resistant to killing by strain HP250. These strains, unlike strain HP250, have a
chromosomally encoded haemocin operon. A number of other members of the family
Pasteurellaceae were tested for haemocin sensitivity. Pasteurella avium, Pasteurella volantium
and Pasteurella species A, all non-pathogenic bacteria found in the respiratory tract of chickens
suffering from respiratory diseases, were sensitive to H. paragallinarum haemocin. However,
amongst the pathogenic Pasteurellaceae, 50 % of P. multocida isolates and all five isolates of
Pasteurella haemolytica tested were sensitive to the haemocin. Given the prevalence of
haemocin production in H. paragallinarum strains, it may play a role in aiding colonization by
inhibiting other Gram-negative bacteria that are associated with the respiratory tract in chickens.
The origin of replication from plasmid p250 has been used to generate an Escherichia coli-H.
paragallinarum shuttle vector which may be useful in genetically manipulating H. paragallinarum.

Xu, J., G. Luo, et al. (2002). “Multiple origins of hybrid strains of Cryptococcus neoformans with serotype
AD.” Microbiology 148(1): 203-212.
http://mic.sgmjournals.org/cgi/content/abstract/148/1/203

Cryptococcus neoformans is a major pathogen of humans throughout the world. Using
commercial mAbs to capsular epitopes, strains of C. neoformans manifest five distinct serotypes -
A, B, C, D and AD. Previous studies demonstrated significant divergence among serotypes A, B,
C and D, which are thought to be haploid. In this study the origins and evolution of strains of
serotype AD were investigated. A portion (537 bp) of the laccase gene was cloned and
sequenced from 14 strains of serotype AD. Each strain contained two different alleles and
sequences for both alleles were obtained. These sequences were compared to those from
serotypes A, B, C and D. This analysis indicated that each of the 14 serotype AD strains
contained two phylogenetically distinct haplotypes: one haplotype was highly similar to the
serotype A group and the other to the serotype D group. To explain the origins of these serotype
AD strains, genealogical analysis is consistent with at least three recent and independent
hybridization events. The results demonstrate that the evolution of C. neoformans is continuing
and dynamic.


http://mic.sgmjournals.org/cgi/content/abstract/149/8/2147

Cryptococcus neoformans is a major pathogen of humans throughout the world. Using commercial monoclonal antibodies to capsular epitopes, strains of C. neoformans manifest five serotypes: A, B, C, D and AD. Previous studies demonstrated significant divergence among serotypes A, B, C and D, which are typically haploid. In contrast, most strains of serotype AD are diploid or aneuploid and result from recent hybridization between strains of serotypes A and D. Whether serotypes A, B, C and D represent strictly asexual lineages is not known. Using comparative genealogical analyses of two genes, the authors investigated whether recombination occurred among strains within serotypes A and D. For each of 14 serotype AD strains, a portion (642 bp) of the orotidine monophosphate pyrophosphorylase (URA5) gene was cloned and sequenced. Each of these 14 strains contained two different alleles and sequences for both alleles were obtained. The URA5 gene genealogy was compared to that derived from the laccase (LAC) gene, which was reported recently for the same 14 strains. For both genes, each of the 14 serotype AD strains contained two phylogenetically distinct alleles: one allele was highly similar to those from serotype A strains and the other to alleles from serotype D strains. However, within both the serotype A allelic group and the serotype D allelic group, there was significant incongruence between genealogies derived from URA5 and LAC. The results suggest recombination in natural populations of both serotypes A and D.


http://mic.sgmjournals.org/cgi/content/abstract/148/8/2351

A gene encoding sorbitol-6-phosphate dehydrogenase (SorF) belonging to the sorbose operon (sorFABCDG) has been characterized in Lactobacillus casei. Inactivation of this gene revealed the presence of another sorbitol-6-phosphate dehydrogenase that was induced by D-sorbitol (D-glucitol). The gene encoding this activity (gutF) has also been isolated, sequenced and disrupted. The sorbitol-6-phosphate dehydrogenase genes (sorF, gutF) were required for growth on L-sorbose and D-sorbitol, respectively. Biochemical and transcriptional analyses of the wild-type and mutant strains demonstrated that L-sorbose and D-sorbitol induced sorF and the gene encoding the sorbose operon activator (sorR), while the expression of gutF was only activated by D-sorbitol. Furthermore, these studies indirectly suggested that a common metabolite of the L-sorbose and D-sorbitol metabolic pathways (probably D-sorbitol 6-phosphate) would act as the effector of SorR. The same effector would also be the inducer of gutF, although the two pathways seem to be subject to distinct regulatory mechanisms.


http://mic.sgmjournals.org/cgi/content/abstract/150/8/2565

The normal microbial flora of the vagina plays an important role in preventing genital and urinary tract infections in women. Thus an accurate understanding of the composition and ecology of the ecosystem is important to understanding the aetiology of these diseases. Common wisdom is that lactobacilli dominate the normal vaginal microflora of post-pubertal women. However, this conclusion is based on methods that require cultivation of microbial populations; an approach that
is known to yield a biased and incomplete assessment of microbial community structure. In this study cultivation-independent methods were used to analyse samples collected from the mid-vagina of five normal healthy Caucasian women between the ages of 28 and 44. Total microbial community DNA was isolated following resuspension of microbial cells from vaginal swabs. To identify the constituent numerically dominant populations in each community 16S rRNA gene libraries were prepared following PCR amplification using the 8f and 926r primers. From each library, the DNA sequences of approximately 200 16S rRNA clones were determined and subjected to phylogenetic analyses. The diversity and kinds of organisms that comprise the vaginal microbial community varied among women. Species of Lactobacillus appeared to dominate the communities in four of the five women. However, the community of one woman was dominated by Atopobium sp., whereas a second woman had appreciable numbers of Megasphaera sp., Atopobium sp. and Leptotrichia sp., none of which have previously been shown to be common members of the vaginal ecosystem. Of the women whose communities were dominated by lactobacilli, there were two distinct clusters, each of which consisted of a single species. One class consisted of two women with genetically divergent clones that were related to Lactobacillus crispatus, whereas the second group of two women had clones of Lactobacillus iners that were highly related to a single phylotype. These surprising results suggest that culture-independent methods can provide new insights into the diversity of bacterial species found in the human vagina, and this information could prove to be pivotal in understanding risk factors for various infectious diseases.


http://mic.sgmjournals.org/cgi/content/abstract/148/6/1833

Lipooligosaccharide (LOS) is a major virulence factor of the pathogenic Neisseria. Nine lgt genes at three chromosomal loci (lgt-1, 2, 3) encoding the glycosyltransferases responsible for the biosynthesis of LOS oligosaccharide chains were examined in 26 Neisseria meningitidis, 51 Neisseria gonorrhoeae and 18 commensal Neisseria strains. DNA hybridization, PCR and nucleotide sequence data were compared to previously reported lgt genes. Analysis of the genetic organization of the lgt loci revealed that in N. meningitidis, the lgt-1 and lgt-3 loci were hypervariable genomic regions, whereas the lgt-2 locus was conserved. In N. gonorrhoeae, no variability in the composition or organization of the three lgt loci was observed. Lgt genes were detected only in some commensal Neisseria species. The genetic organization of the lgt-1 locus was classified into eight types and the lgt-3 locus was classified into four types. Two types of arrangement at lgt-1 (II and IV) and one type of arrangement at lgt-3 (IV) were novel genetic organizations reported in this study. Based on the three lgt loci, 10 LOS genotypes of N. meningitidis were distinguished. Phylogenetic analysis revealed a gene cluster, lgtH, which separated from the homologous genes lgtB and lgtE. The lgtH and lgtE genes were mutually exclusive and were located at the same position in lgt-1. The data demonstrated that pathogenic and commensal Neisseria share a common lgt gene pool and horizontal gene transfer appears to contribute to the genetic diversity of the lgt loci in Neisseria.

Microchemical Journal (1)

We have investigated the effect of polymer gel reconditioning, the shape of the capillary, the applied electric field, and the capillary length for single-stranded DNA. The polyethylene oxide gel had deformed under the high electric field causing the degradation of the separation power. By the reintroduction of the fresh polyethylene oxide gel for the next run, one-base resolution was recovered. It turned out that the tip of the capillary at the injection side needed to be clean and symmetric for much improved resolution. Changing DNA motion by the pulsed electric field resulted in the separation of DNA far more than 500 bases.

**Mitochondrion (5)**


http://www.sciencedirect.com/science/article/B6W8G-4DKGXH1-1/2/849c94362428da6333e4468400d4a458

It was hypothesised that mitochondrial iron overload in patients with refractory anemia with ring sideroblasts (RARS) results from mitochondrial DNA (mtDNA) mutations. To analyse the mtDNA sequence of iron storing mitochondria sensitively, we developed new protocols for selective erythroblasts isolation, mtDNA PCR amplification and sequencing. Using this approach, we found in each of the three RARS patients examined a unique spectrum of homoplasmic mtDNA point mutations affecting several mtDNA genes. Prediction analyses suggest that identified mutations do not result in major perturbations of mitochondrial functions and are tolerated. We discuss a mechanism explaining how the mutations identified may contribute to RARS pathogenesis.


Forensic and clinical laboratories benefit from DNA standard reference materials (SRMs) that provide the quality control and assurance that their results from sequencing unknown samples are correct. Therefore, the mitochondrial DNA (mtDNA) genome of HL-60, a promyelocytic leukemia cell line, has been completely sequenced by four laboratories and will be available to the forensic and medical communities in the spring of 2003; it will be called National Institute of Standards and Technology (NIST) SRM 2392-I. NIST human mtDNA SRM 2392 will continue to be available and includes the DNA from two apparently healthy individuals. Both SRM 2392 and 2392-I contain all the information (e.g. the sequences of 58 unique primer sets) needed to use
These SRMs as positive controls for the amplification and sequencing any DNA. Compared to the templates in SRM 2392, the HL-60 mtDNA in SRM 2392-I has two tRNA differences and more polymorphisms resulting in amino acid changes. Four of these HL-60 mtDNA polymorphisms have been associated with Leber Hereditary Optic Neuropathy (LHON), one as an intermediate mutation and three as secondary mutations. The mtDNA from a cell line (GM10742A) from an individual with LHON was also completely sequenced for comparison and contained some of the same LHON mutations. The combination of these particular LHON associated mutations is also found in phylogenetic haplogroup J and its subset, J2, and may only be indicative that HL-60 belongs to haplogroup J, one of nine haplogroups that characterize Caucasian individuals of European descent or may mean that haplogroup J is more prone to LHON. Both these mtDNA SRMs will provide enhanced quality control in forensic identification, medical diagnosis, and single nucleotide polymorphism detection.


http://www.sciencedirect.com/science/article/B6W8G-4908C03-3/2/3c31ca42d7c99ecd6fd8cea0fe83083c

This study describes a multiplex real-time polymerase chain reaction (PCR) assay that quantifies total mitochondrial DNA (mtDNAtotal) and mtDNA bearing the 4977-base pair 'common deletion' ([Delta]mtDNA4977) in lymphoblasts derived from an individual diagnosed with Pearson's syndrome. The method is unique in its use of plasmids as external quantification standards and its use of multiplex conditions. Standards are validated by comparison with purified mtDNA amplification curves and by the fact that curves are largely unaffected by nuclear DNA (nucDNA). Finally, slopes of standard curves and unknowns are shown to be similar to each other and to theoretical predictions. From these data, mtDNAtotal in these cells is calculated to be 3258 (+723/-592) copies per cell while [Delta]mtDNA4977 averages 232 (+136/-86) copies per cell or 7% (+4.65/-2.81).


http://www.sciencedirect.com/science/article/B6W8G-488Y3TT-1/2/e49410319a737974d52d33abc7a20711

We investigated the molecular relationships between lipid peroxidation and mitochondrial DNA (mtDNA) single strand breaks (ssb) in isolated rat hepatocytes and mitochondria exposed to tert-butylhydroperoxide (TBH). Our results show that mtDNA ssb induced by TBH are independent of lipid peroxidation and dependent on the presence of iron and of hydroxyl free radicals. These data contribute to the definition of the mechanisms whereby mtDNA ssb are induced and provide possible molecular targets for the prevention of this kind of damage in vivo.

The transmission of a C16,291C/T heteroplasmy in the HV1 region of human mitochondrial DNA (mtDNA) was examined in buccal cells from 13 maternally-related individuals across three generations and in additional tissues (hair, blood, or finger nails) from three members of this family. The ratio of C:T at nucleotide position (np) 16,291 showed wide intra- and intergenerational variation as well as tissue variation within individuals. Our results demonstrate that one or two sequence differences between samples in the mtDNA does not warrant an exclusion. To avoid false exclusions especially when comparing mtDNA from hair samples, we recommend the analysis of as many samples as possible in order to minimize the possibility that the detection of a rare polymorphism in a single sample would be considered an exclusion when it is really a match. The observation that the transmission of a mtDNA heteroplasmy from one individual to her offspring is likely to differ among the first-generation offspring and between that generation and subsequent generations lends further credence to the bottleneck theory of inheritance of human mtDNA.

Mol Ther (1)


Real-time PCR is a powerful method for the quantification of gene expression in biological samples. This method uses TaqMan chemistry based on the 5' -exonuclease activity of the AmpliTaq Gold DNA polymerase which releases fluorescence from hybridized probes during synthesis of each new PCR product. Many gene therapy studies use lacZ, encoding Escherichia coli beta-galactosidase, as a marker gene. Our results demonstrate that E. coli DNA contamination in AmpliTaq Gold polymerase interferes with TaqMan analysis of lacZ gene expression and decreases sensitivity of the method below the level required for biodistribution and long-term gene expression studies. In biodistribution analyses the contamination can lead to false-negative results by masking low-level lacZ expression in target and ectopic tissues, and false-positive results if sufficient controls are not used. We conclude that, to get reliable TaqMan results with lacZ, adequate controls should be included in each run to rule out contamination from AmpliTaq Gold polymerase.

Mol. Biol. Cell (10)

The small GTPase Ran has been found to play pivotal roles in several aspects of cell function. We have investigated the role of the Ran GTPase cycle in spindle formation and nuclear envelope assembly in dividing Caenorhabditis elegans embryos in real time. We found that Ran and its cofactors RanBP2, RanGAP, and RCC1 are all essential for reformation of the nuclear envelope after cell division. Reducing the expression of any of these components of the Ran GTPase cycle by RNAi leads to strong extranuclear clustering of integral nuclear envelope proteins and nucleoporins. Ran, RanBP2, and RanGAP are also required for building a mitotic spindle, whereas astral microtubules are normal in the absence of these proteins. RCC1(RNAi) embryos have similar abnormalities in the initial phase of spindle formation but eventually recover to form a bipolar spindle. Irregular chromatin structures and chromatin bridges due to spindle failure were frequently observed in embryos where the Ran cycle was perturbed. In addition, connection between the centrosomes and the male pronucleus, and thus centrosome positioning, depends upon the Ran cycle components. Finally, we have demonstrated that both IMA-2 and IMB-1, the homologues of vertebrate importin [alpha] and [beta], are essential for both spindle assembly and nuclear formation in early embryos.


We have shown previously that the transforming growth factor-[beta] (TGF[beta])-regulated Smad proteins transactivate the apolipoprotein C-III promoter in hepatic cells via a hormone response element that binds the nuclear receptor hepatocyte nuclear factor 4 (HNF-4). In the present study, we show that Smad3 and Smad4 but not Smad2 physically interact with HNF-4 via their Mad homology 1 domains both in vitro and in vivo. The synergistic transactivation of target promoters by Smads and HNF-4 was shown to depend on the specific promoter context and did not require an intact [beta]-hairpin/DNA binding domain of the Smads. Using glutathione S-transferase interaction assays, we established that two regions of HNF-4, the N-terminal activation function 1 (AF-1) domain (aa 1-24) and the C-terminal F domain (aa 388-455) can mediate physical Smad3/HNF-4 interactions in vitro. In vivo, Smad3 and Smad4 proteins enhanced the transactivation function of various GAL4-HNF-4 fusion proteins via the AF-1 and the adjacent DNA binding domain, whereas a single tyrosine to alanine substitution in AF-1 abolished coactivation by Smads. The findings suggest that the transcriptional cross talk between the TGF[beta]-regulated Smads and HNF-4 is mediated by specific functional domains in the two types of transcription factors. Furthermore, the specificity of this interaction for certain target promoters may play an important role in various hepatocyte functions, which are regulated by TGF[beta] and the Smads.


Fungal sphingolipids contain ceramide with a very-long-chain fatty acid (C26). To investigate the physiological significance of the C26-substitution on this lipid, we performed a screen for mutants that are synthetically lethal with ELO3. Elo3p is a component of the ER-associated fatty acid
elongase and is required for the final elongation cycle to produce C26 from C22/C24 fatty acids. elo3[Δ] mutant cells thus contain C22/C24 instead of the natural C26-substituted ceramide. We now report that under these conditions, an otherwise nonessential, but also fungal-specific, structural modification of the major sterol of yeast, ergosterol, becomes essential, because mutations in ELO3 are synthetically lethal with mutations in ERG6. Erg6p catalyzes the methylation of carbon atom 24 in the aliphatic side chain of sterol. The lethality of an elo3[Δ] erg6[Δ] double mutant is rescued by supplementation with ergosterol but not with cholesterol, indicating a vital structural requirement for the ergosterol-specific methyl group. To characterize this structural requirement in more detail, we generated a strain that is temperature sensitive for the function of Erg6p in an elo3[Δ] mutant background. Examination of raft association of the GPI-anchored Gas1p and plasma membrane ATPase, Pma1p, in the conditional elo3[Δ] erg6ts double mutant, revealed a specific defect of the mutant to maintain raft association of preexisting Pma1p. Interestingly, in an elo3[Δ] mutant at 37°C, newly synthesized Pma1p failed to enter raft domains early in the biosynthetic pathway, and upon arrival at the plasma membrane was rerouted to the vacuole for degradation. These observations indicate that the C26 fatty acid substitution on lipids is important for establishing raft association of Pma1p and stabilizing the protein at the cell surface. Analysis of raft lipids in the conditional mutant strain revealed a selective enrichment of ergosterol in detergent-resistant membrane domains, indicating that specific structural determinants on both sterols and sphingolipids are required for their association into raft domains.


Monitoring Editor: Tim Stearns

The fission yeast Mcs6-Mcs2-Pmh1 complex, homologous to metazoan Cdk7-cyclin H-Mat1, has dual functions in cell division and transcription: as a partially redundant CDK-activating kinase (CAK) that phosphorylates the major cell-cycle CDK, Cdc2, on Thr-167; and as the RNA polymerase (Pol) II carboxyl-terminal domain (CTD) kinase associated with transcription factor (TF) IIH. We analyzed conditional mutants of mcs6 and pmh1, which activate Cdc2 normally but cannot complete cell division at restrictive temperature and arrest with decreased CTD phosphorylation. Transcriptional profiling by microarray hybridization revealed only modest effects on global gene expression: a one-third reduction in a severe mcs6 mutant after prolonged incubation at 36°C. In contrast, a small subset of transcripts (≤5%) decreased by >2-fold after Mcs6-complex function was compromised. The signature of repressed genes overlapped significantly with those of cell-separation mutants sep10 and sep15. Sep10, a component of the Pol II Mediator complex, becomes essential in mcs6 or pmh1 mutant backgrounds. Moreover, transcripts dependent on the forkhead transcription factor Sep1, which are expressed coordinately during mitosis, were repressed in Mcs6-complex mutants, and Mcs6 also interacts genetically with Sep1. Thus the Mcs6 complex, a direct activator of Cdc2, also influences the cell-cycle transcriptional program, possibly through its TFIIH-associated kinase function.


The Eph family of receptor tyrosine kinases regulates numerous biological processes. To examine the biochemical and developmental contributions of specific structural motifs within Eph receptors, wild-type or mutant forms of the EphA4 receptor were ectopically expressed in
developing Xenopus embryos. Wild-type EphA4 and a mutant lacking both the SAM domain and PDZ binding motif were constitutively tyrosine phosphorylated in vivo and catalytically active in vitro. EphA4 induced loss of cell adhesion, ventro-lateral protrusions, and severely expanded posterior structures in Xenopus embryos. Moreover, mutation of a conserved SAM domain tyrosine to phenylalanine (Y928F) enhanced the ability of EphA4 to induce these phenotypes, suggesting that the SAM domain may negatively regulate some aspects of EphA4 activity in Xenopus. Analysis of double mutants revealed that the Y928F EphA4 phenotypes were dependent on kinase activity; juxtamembrane sites of tyrosine phosphorylation and SH2 domain-binding were required for cell dissociation, but not for posterior protrusions. The induction of protrusions and expansion of posterior structures is similar to phenotypic effects observed in Xenopus embryos expressing activated FGFR1. Furthermore, the budding ectopic protrusions induced by EphA4 express FGFR-8, FGFR1, and FGFR4a. In addition, antisense morpholino oligonucleotide-mediated loss of FGFR-8 expression in vivo substantially reduced the phenotypic effects in EphA4Y928F expressing embryos, suggesting a connection between Eph and FGF signaling.


http://www.molbiolcell.org/cgi/content/abstract/15/1/24

In the budding yeast Saccharomyces cerevisiae, phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2) is synthesized by a single phosphatidylinositol 3-phosphate 5-kinase, Fab1. Cells deficient in PtdIns(3,5)P2 synthesis exhibit a grossly enlarged vacuole morphology, whereas increased levels of PtdIns(3,5)P2 provokes the formation of multiple small vacuoles, suggesting a specific role for PtdIns(3,5)P2 in vacuole size control. Genetic studies have indicated that Fab1 kinase is positively regulated by Vac7 and Vac14; deletion of either gene results in ablation of PtdIns(3,5)P2 synthesis and the formation of a grossly enlarged vacuole. More recently, a suppressor of vac7{Delta} mutants was identified and shown to encode a putative phosphoinositide phosphatase, Fig4. We demonstrate that Fig4 is a magnesium-activated PtdIns(3,5)P2-selective phosphoinositide phosphatase in vitro. Analysis of a Fig4-GFP fusion protein revealed that the Fig4 phosphatase is localized to the limiting membrane of the vacuole. Surprisingly, in the absence of Vac14, Fig4-GFP no longer localizes to the vacuole. However, Fig4-GFP remains localized to the grossly enlarged vacuoles of vac7 deletion mutants. Consistent with these observations, we found that Fig4 physically associates with Vac14 in a common membrane-associated complex. Our studies indicate that Vac14 both positively regulates Fab1 kinase activity and directs the localization/activation of the Fig4 PtdIns(3,5)P2 phosphatase.


http://www.molbiolcell.org/cgi/content/abstract/16/2/776

Phosphorylated derivatives of phosphatidylinositol are essential regulators of both endocytic and exocytic trafficking in eukaryotic cells. In Saccharomyces cerevisiae, the phosphatidylinositol 4-kinase, Pik1p generates a distinct pool of PtdIns(4)P that is required for normal Golgi structure and secretory function. Here, we utilize a synthetic genetic array analysis of a conditional pik1 mutant to identify candidate components of the Pik1p/PtdIns(4)P signaling pathway at the Golgi. Our data suggest a mechanistic involvement for Pik1p with a specific subset of Golgi-associated
proteins, including the Ypt31p rab-GTPase and the TRAPPII protein complex, to regulate protein trafficking through the secretory pathway. We further demonstrate that TRAPPII specifically functions in a Ypt31p-dependent pathway and identify Gyp2p as the first biologically relevant GTPase activating protein for Ypt31p. We propose that multiple stage-specific signals, which may include Pik1p/PtdIns(4)P, TRAPPII and Gyp2p, impinge upon Ypt31 signaling to regulate Golgi secretory function.


http://www.molbiolcell.org/cgi/content/abstract/15/8/3591

Ligand binding causes the EGF receptor (EGFR) to become ubiquitinated by Cbl upon association with the adaptor protein Grb2. We have investigated the role of ubiquitin and Grb2 in ligand-induced endocytosis of the EGFR. Incubation of cells with EGF on ice caused translocation of Grb2 and Cbl from the cytosol to the rim of coated pits. Grb2 with point mutations in both SH3 domains inhibited recruitment of the EGFR to clathrin-coated pits, in a Ras-independent manner. On overexpression of the Cbl-binding protein Sprouty, ubiquitination of the EGFR was inhibited, the EGFR was recruited only to the rim of coated pits, and endocytosis of the EGFR was inhibited. Conjugation-defective ubiquitin similarly inhibited recruitment of EGF-EGFR to clathrin-coated pits. Even though this does not prove that cargo must be ubiquitinated, this indicates the importance of interaction of ubiquitinated protein(s) with proteins harboring ubiquitin-interacting domains. We propose that Grb2 mediates transient anchoring of the EGFR to an Eps15-containing molecular complex at the rim of coated pits and that Cbl-induced ubiquitination of the EGFR allows relocation of EGFR from the rim to the center of clathrin-coated pits.


http://www.molbiolcell.org/cgi/content/abstract/13/12/4429

The degree of acyl chain desaturation of membrane lipids is a critical determinant of membrane fluidity. Temperature-sensitive mutants of the single essential acyl chain desaturase, Ole1p, of yeast have previously been isolated in screens for mitochondrial inheritance mutants (Stewart, L.C., and Yaffe, M.P. (1991). J. Cell Biol. 115, 1249-1257). We now report that the mutant desaturase relocalizes from its uniform ER distribution to a more punctuate localization at the cell periphery upon inactivation of the enzyme. This relocalization takes place within minutes at nonpermissive conditions, a time scale at which mitochondrial morphology and inheritance is not yet affected. Relocalization of the desaturase is fully reversible and does not affect the steady state localization of other ER resident proteins or the kinetic and fidelity of the secretory pathway, indicating a high degree of selectivity for the desaturase. Relocalization of the desaturase is energy independent but is lipid dependent because it is rescued by supplementation with unsaturated fatty acids. Relocalization of the desaturase is also observed in cells treated with inhibitors of the enzyme, indicating that it is independent of temperature-induced alterations of the enzyme. In the absence of desaturase function, lipid synthesis continues, resulting in the generation of lipids with saturated acyl chains. A model is discussed in which the accumulation of saturated lipids in a microdomain around the desaturase could induce the observed segregation and relocalization of the enzyme.

http://www.molbiolcell.org/cgi/content/abstract/13/3/1058

The subpellicular microtubules of the trypanosome cytoskeleton are cross-linked to each other and the plasma membrane, creating a cage-like structure. We have isolated, from Trypanosoma brucei, two related low-molecular-weight cytoskeleton-associated proteins (15- and 17-kDa), called CAP15 and CAP17, which are differentially expressed during the life cycle. Immunolabeling shows a corset-like colocalization of both CAPs and tubulin. Western blot and electron microscope analyses show CAP15 and CAP17 labeling on detergent-extracted cytoskeletons. However, the localization of both proteins is restricted to the anterior, microtubule minus, and less dynamic half of the corset. CAP15 and CAP17 share properties of microtubule-associated proteins when expressed in heterologous cells (Chinese hamster ovary and HeLa), colocalization with their microtubules, induction of microtubule bundle formation, cold resistance, and insensitivity to nocodazole. When overexpressed in T. brucei, both CAP15 and CAP17 cover the whole subpellicular corset and induce morphological disorders, cell cycle-based abnormalities, and subsequent asymmetric cytokinesis.

Mol. Biol. Evol. (19)


http://mbe.oupjournals.org/cgi/content/abstract/20/1/54

Genealogies generated through a long-term study of superb fairy-wrens (Malurus cyaneus) were used to investigate mutation within two hypervariable microsatellite loci. Of 3,230 meioses examined at the tetrancleotide locus (Mcy{micro}8), 45 mutations were identified, giving a mutation rate of 1.4%. At the dinucleotide locus (Mcy{micro}4) 30 mutations were recorded from 2,750 meioses giving a mutation rate of 1.1%. Mutations at both loci primarily (80%; 60/75) involved the loss or gain of a single repeat unit. Unlike previous studies, there was no significant bias toward additions over deletions. The mutation rate at Mcy{micro}8 increased with allele size, and very long alleles (>70 repeats) mutated at a rate of almost 20%. The length of the mutating allele and allele span, however, were strongly correlated so it was not possible to isolate the causative factor. Allele size did not appear to affect mutation rate at Mcy{micro}4, but the repeat number was considerably lower at this locus. The gender of the mutating parent was significant only at Mcy{micro}8, where mutations occurred more frequently in maternal alleles. However, at both loci we found that alleles inherited from the mother were on average larger than those from the father, and this in part drove the higher mutation rate among maternally inherited alleles at Mcy{micro}8.


http://mbe.oupjournals.org/cgi/content/abstract/19/12/2176
Recent adaptive radiations provide excellent model systems for understanding speciation, but rapid diversification can cause problems for phylogenetic inference. Here we use gene genealogies to investigate the phylogeny of recent speciation in the heliconiine butterflies. We sequenced three gene regions, intron 3 (approx.550 bp) of sex-linked triose-phosphate isomerase (Tpi), intron 3 (approx.450 bp) of autosomal mannose-phosphate isomerase (Mpi), and 1,603 bp of mitochondrial cytochrome oxidase subunits I and II (COI and COII), for 37 individuals from 25 species of Heliconius and related genera. The nuclear intron sequences evolved at rates similar to those of mitochondrial coding sequences, but the phylogenetic utility of introns was restricted to closely related geographic populations and species due to high levels of indel variation. For two sister species pairs, Heliconius erato-Heliconius himera and Heliconius melpomene-Heliconius cydno, there was highly significant discordance between the three genes. At mtDNA and Tpi, the hypotheses of reciprocal monophyly and paraphyly of at least one species with respect to its sister could not be distinguished. In contrast alleles sampled from the third locus, Mpi, showed polyphyletic relationships between both species pairs. In all cases, recent coalescence of mtDNA lineages within species suggests that polyphyly of nuclear genes is not unexpected. In addition, very similar alleles were shared between melpomene and cydno, implying recent gene flow. Our finding of discordant genealogies between genes is consistent with models of adaptive speciation with ongoing gene flow and highlights the need for multiple locus comparisons to resolve phylogeny among closely related species.


http://mbe.oupjournals.org/cgi/content/abstract/20/4/541

Ecdysteroid hormones are major regulators in reproduction and development of insects, including larval molts and metamorphosis. The functional ecdysone receptor is a heterodimer of ECR (NR1H1) and USP-RXR (NR2B4), which is the orthologue of vertebrate retinoid X receptors (RXR {alpha}, {beta}, {gamma}). Both proteins belong to the superfamily of nuclear hormone receptors, ligand-dependent transcription factors that share two conserved domains: the DNA-binding domain (DBD) and the ligand-binding domain (LBD). In order to gain further insight into the evolution of metamorphosis and gene regulation by ecdysone in arthropods, we performed a phylogenetic analysis of both partners of the heterodimer ECR/USP-RXR. Overall, 38 USP-RXR and 19 ECR protein sequences, from 33 species, have been used for this analysis. Interestingly, sequence alignments and structural comparisons reveal high divergence rates, for both ECR and USP-RXR, specifically among Diptera and Lepidoptera. The most impressive differences affect the ligand-binding domain of USP-RXR. In addition, ECR sequences show variability in other domains, namely the DNA-binding and the carboxy-terminal F domains. Our data provide the first evidence that ECR and USP-RXR may have coevolved during holometabolous insect diversification, leading to a functional divergence of the ecdysone receptor. These results have general implications on fundamental aspects of insect development, evolution of nuclear receptors, and the design of specific insecticides.


http://mbe.oupjournals.org/cgi/content/abstract/19/11/1870

Population-level studies using the major histocompatibility complex (Mhc) have linked specific alleles with specific diseases, but data requirements are high and the power to detect disease association is low. A novel use of Mhc population surveys involves mapping allelic substitutions
onto the inferred structural molecular model to show functional differentiation related to local selective pressures. In the estuarine fish Fundulus heteroclitus, populations experiencing strong differences in antigenic challenges show significant differences in amino acid substitution patterns that are reflected as variation in the structural location of changes between populations. Fish from a population genetically adapted to severe chemical pollution also show novel patterns of DNA substitution at a highly variable Mhc class II B locus including strong signals of positive selection at inferred antigen-binding sites and population-specific signatures of amino acid substitution. Heavily parasitized fish from an extreme PCB-contaminated (U.S. Environmental Protection Agency Superfund) site show enhanced population-specific substitutions in the a-helix portion of the inferred antigen-binding region. In contrast, fish from an unpolluted site show a significantly different pattern focused on the first strand of the B-pleated sheet. Whether Mhc population profile differences represent the direct effects of chemical toxicants or indirect parasite-mediated selection, the result is a composite habitat-specific signature of strong selection and evolution affecting the genetic repertoire of the major histocompatibility complex.


http://mbe.oupjournals.org/cgi/content/abstract/21/9/1712

Centromeric DNA, being highly repetitive, has been refractory to molecular analysis. However, centromeric structural proteins are encoded by single-copy genes, and these can be analyzed by using standard phylogenetic tools. The centromere-specific histone, CenH3, replaces histone H3 in centromeric nucleosomes, and is required for the proper distribution of chromosomes during cell division. Whereas histone H3s are nearly identical between species, CenH3s are divergent, with an N-terminal tail that is highly variable in length and sequence. Both the N-terminal tail and histone fold domain (HFD) are subject to adaptive evolution in Drosophila. Similarly, comparisons between Arabidopsis thaliana and Arabidopsis arenosa detected adaptive evolution, but only in the N-terminal tail. We have extended our evolutionary analyses of CenH3s to other members of the Brassicaceae, which allowed the detection of positive selection in both the N-terminal tail and in the HFD. We find that adaptively evolving sites in the HFD can potentially interact with DNA, including sites in the loop 1 region of the HFD that are required for centromeric targeting in Drosophila. Other adaptively evolving sites in the HFD can be localized on the structure of the nucleosome core particle, revealing an extended surface in addition to loop 1 in which conformational changes might alter histone-DNA contacts or water bridges. The identification of adaptively evolving sites provides a structural basis for the interaction between centromeric DNA and the protein that is thought to underlie the evolution of centromeres and the accumulation of pericentric heterochromatin.


http://mbe.oupjournals.org/cgi/content/abstract/19/2/179

Nucleotide polymorphism in Scots pine (Pinus sylvestris) was studied in the gene encoding phenylalanine ammonia-lyase (Pal, EC 4.3.1.5). Scots pine, like many other pine species, has a large current population size. The observed levels of inbreeding depression suggest that Scots pine may have a high mutation rate to deleterious alleles. Many Scots pine markers such as isozymes, RFLPs, and microsatellites are highly variable. These observations suggest that the levels of nucleotide variation should be higher than those in other plant species. A 2,045-bp fragment of the pal1 locus was sequenced from five megagametophytes each from a different individual from each of four populations, from northern and southern Finland, central Russia, and
northern Spain. There were 12 segregating sites in the locus. The synonymous site overall nucleotide diversity was only 0.0049. In order to compare pal1 with other pine genes, sequence was obtained from two alleles of 11 other loci (total length 4,606 bp). For these, the synonymous nucleotide diversity was 0.0056. These estimates are lower than those from other plants. This is most likely because of a low mutation rate, as estimated from between-pine species synonymous site divergence. In other respects, Scots pine has the characteristics of a species with a large effective population. There was no linkage disequilibrium even between closely linked sites. This resulted in high haplotype diversity (14 different haplotypes among 20 sequences). This could also give rise to high per locus diversity at the protein level. Divergence between populations in the main range was low, whereas an isolated Spanish population had slightly lower diversity and higher divergence than the remaining populations.


http://mbe.oupjournals.org/cgi/content/abstract/21/5/799

To characterize patterns of genomic variation in central chimpanzees (Pan troglodytes troglodytes) and gain insight into their evolution, we sequenced nine unlinked, intergenic regions, representing a total of 19,000 base pairs, in 14 individuals. When these DNA sequences are compared with homologous sequences previously collected in humans and in western chimpanzees (Pan troglodytes verus), nucleotide diversity is higher in central chimpanzees than in western chimpanzees or in humans. Consistent with a larger effective population size of central chimpanzees, levels of linkage disequilibrium are lower than in humans. Patterns of linkage disequilibrium further suggest that homologous gene conversion may be an important contributor to genetic exchange at short distances, in agreement with a previous study of the same DNA sequences in humans. In central chimpanzees, but not in western chimpanzees, the allele frequency spectrum is significantly skewed towards rare alleles, pointing to population size changes or fine-scale population structure. Strikingly, the extent of genetic differentiation between western and central chimpanzees is much stronger than what is seen between human populations. This suggests that careful attention should be paid to geographic sampling in studies of chimpanzee genetic variation.


http://mbe.oupjournals.org/cgi/content/abstract/21/5/781

The diversity, origin, and evolution of chromoviruses in Eukaryota were examined using the massive amount of genome sequence data for different eukaryotic lineages. A surprisingly large number of novel full-length chromoviral elements were found, greatly exceeding the number of the known chromoviruses. These new elements are mostly structurally intact and highly conserved. Chromoviruses in the key Amniota lineage, the reptiles, have been analyzed by PCR to explain their evolutionary dynamics in amniotes. Phylogenetic analyses provide evidence for a novel centromere-specific chromoviral clade that is widespread and highly conserved in all seed plants. Chromoviral diversity in plants, fungi, and vertebrates, as shown by phylogenetic analyses, was found to be much greater than previously expected. The age of plant chromoviruses has been significantly extended by finding their representatives in the most basal plant lineages, the green and the red algae. The evolutionary origin of chromoviruses has been found to be no earlier than in Cercozoa. The evolutionary history and dynamics of chromoviruses can be explained simply by strict vertical transmission in plants, followed by more complex evolution in fungi and in Metazoa. The currently available data clearly show that chromoviruses
indeed represent the oldest and the most widespread clade of Metaviridae.


http://mbe.oupjournals.org/cgi/content/abstract/21/1/158

Sex chromosomes provide a useful context for the study of the relative importance of evolutionary forces affecting genetic diversity. The human Y chromosome shows levels of nucleotide diversity 20% that of autosomes, which is significantly less than expected when differences in effective population size and sex-specific mutation rates are taken into account. To study the generality of low levels of Y chromosome variability in mammalian genomes, we investigated nucleotide diversity in intron sequences of X (1.1-3.0 kb) and Y (0.7-3.5 kb) chromosome genes of five mammals: lynx, wolf, reindeer, cattle, and field vole. For all species, nucleotide diversity was found to be lower on Y than on X, with no segregating site observed in Y-linked sequences of lynx, reindeer, and cattle. For X chromosome sequences, nucleotide diversity was in the range of 1.6 x 10^{-4} (lynx) to 8.0 x 10^{-4} (field vole). When differences in effective population size and the extent of the male mutation bias were taken into account, all five species showed evidence of reduced levels of Y chromosome variability. Reduced levels of Y chromosome variability have also been observed in Drosophila and in plants, as well as in the female-specific W chromosome of birds. Among the different factors proposed to explain low levels of genetic variability in the sex-limited chromosome (Y/W), we note that selection is the only factor that is broadly applicable irrespective of mode of reproduction and whether there is male or female heterogamety.


http://mbe.oupjournals.org/cgi/content/abstract/19/8/1244

Cave bears (Ursus spelaeus) existed in Europe and western Asia until the end of the last glaciation some 10,000 years ago. To investigate the genetic diversity, population history, and relationship among different cave bear populations, we have determined mitochondrial DNA sequences from 12 cave bears that range in age from about 26,500 to at least 49,000 years and originate from nine caves. The samples include one individual from the type specimen population, as well as two small-sized high-Alpine bears. The results show that about 49,000 years ago, the mtDNA diversity among cave bears was about 1.8-fold lower than the current species-wide diversity of brown bears (Ursus arctos). However, the current brown bear mtDNA gene pool consists of three clades, and cave bear mtDNA diversity is similar to the diversity observed within each of these clades. The results also show that geographically separated populations of the high-Alpine cave bear form were polyphyletic with respect to their mtDNA. This suggests that small size may have been an ancestral trait in cave bears and that large size evolved at least twice independently.


http://mbe.oupjournals.org/cgi/content/abstract/21/3/454
The Indian subcontinent contains 20 well-characterized goat breeds, which vary in their genetic potential for the production of milk, meat, and fibre; disease resistance; heat tolerance; and fecundity. Indian goats make up 20% of the world's goat population, but there has been no extensive study of these economically important animals. Therefore, we have undertaken the present investigation of 363 goats belonging to 10 different breeds from different geographic regions of India using mtDNA sequence data from the HVRI region. We find evidence for population structure and novel lineages in Indian goats and cannot reconcile the genetic diversity found within the major lineage with domestication starting 10,000 years ago from a single mtDNA ancestor. Thus, we propose a more complex origin for domestic goats.


http://mbe.oupjournals.org/cgi/content/abstract/21/5/936

We humans have many characteristics that are different from those of the great apes. These human-specific characters must have arisen through mutations accumulated in the genome of our direct ancestor after the divergence of the last common ancestor with chimpanzee. Gene trees of human and great apes are necessary for extracting these human-specific genetic changes. We conducted a systematic analysis of 103 protein-coding genes for human, chimpanzee, gorilla, and orangutan. Nucleotide sequences for 18 genes were newly determined for this study, and those for the remaining genes were retrieved from the DDBJ/EMBL/GenBank database. The total number of amino acid changes in the human lineage was 147 for 26,199 codons (0.56%). The total number of amino acid changes in the human genome was, thus, estimated to be about 80,000. We applied the acceleration index test and Fisher's synonymous/nonsynonymous exact test for each gene tree to detect any human-specific enhancement of amino acid changes compared with ape branches. Six and two genes were shown to have significantly higher nonsynonymous changes at the human lineage from the acceleration index and exact tests, respectively. We also compared the distribution of the differences of the nonsynonymous substitutions on the human lineage and those on the great ape lineage. Two genes were more conserved in the ape lineage, whereas one gene was more conserved in the human lineage. These results suggest that a small proportion of protein-coding genes started to evolve differently in the human lineage after it diverged from the ape lineage.


http://mbe.oupjournals.org/cgi/content/abstract/20/8/1281

We have investigated the pattern and extent of nucleotide diversity in 10 X-chromosomal genes where mutations are known to cause mental retardation in humans. For each gene, we sequenced the entire coding region from cDNA in humans, chimpanzees, and orangutans, as well as about 3 kb of genomic DNA in 20 humans sampled worldwide and in 10 chimpanzees representing two “subspecies.” Overall nucleotide diversity in these genes is about twofold lower in humans than in chimpanzees, and nucleotide diversity within and between species is low, suggesting that a high level of functional constraint acts on these genes. Strikingly, we find that a summary of the allele frequency spectrum is significantly correlated in humans and chimpanzees, perhaps reflecting very similar levels of constraint at these genes in the two species. A possible exception is FMR2, which shows a higher number of nonsynonymous than synonymous substitutions on the human lineage, suggesting the action of positive selection.
Bindin is a gamete recognition protein known to control species-specific sperm-egg adhesion and membrane fusion in sea urchins. Previous analyses have shown that diversifying selection on bindin amino acid sequence is found when gametically incompatible species are compared, but not when species are compatible. The present study analyzes bindin polymorphism and divergence in the three closely related species of Echinometra in Central America: E. lucunter and E. viridis from the Caribbean, and E. vanbrunti from the eastern Pacific. The eggs of E. lucunter have evolved a strong block to fertilization by sperm of its neotropical congeners, whereas those of the other two species have not. As in the Indo-West Pacific (IWP) Echinometra, the neotropical species show high intraspecific bindin polymorphism in the same gene regions as in the IWP species. Maximum likelihood analysis shows that many of the polymorphic codon sites are under mild positive selection. Of the fixed amino acid replacements, most have accumulated along the bindin lineage of E. lucunter. We analyzed the data with maximum likelihood models of variation in positive selection across lineages and codon sites, and with models that consider sites and lineages simultaneously. Our results show that positive selection is concentrated along the E. lucunter bindin lineage, and that codon sites with amino acid replacements fixed in this species show by far the highest signal of positive selection. Lineage-specific positive selection paralleling egg incompatibility provides support that adaptive evolution of sperm proteins acts to maintain recognition of bindin by changing egg receptors. Because both egg incompatibility and bindin divergence are greater between allopatric species than between sympatric species, the hypothesis of selection against hybridization (reinforcement) cannot explain why adaptive evolution has been confined to a single lineage in the American Echinometra. Instead, processes acting to varying degrees within species (e.g., sperm competition, sexual selection, and sexual conflict) are more promising explanations for lineage-specific positive selection on bindin.


The Atlantic auk assemblage includes four extant species, razorbill (Alca torda), dovekie (Alle alle), common murre (Uria aalge), and thick-billed murre (U. lomvia), and one recently extinct species, the flightless great auk (Pinguinus impennis). To determine the phylogenetic relationships among the species, a contiguous 4.2-kb region of the mitochondrial genome from the extant species was amplified using PCR. This region included one ribosomal RNA gene, four transfer RNA genes, two protein-coding genes, the control region, and intergenic spacers. Sets of PCR primers for amplifying the same region from great auk were designed from sequences of the extant species. The authenticity of the great auk sequence was ascertained by alternative amplifications, cloning, and separate analyses in an independent laboratory. Phylogenetic analyses of the entire assemblage, made possible by the great auk sequence, fully resolved the phylogenetic relationships and split it into two primary lineages, Uria versus Alle, Alca, and Pinguinus. A sister group relationship was identified between Alca and Pinguinus to the exclusion of Alle. Phylogenetically, the flightless great auk originated late relative to other divergences within the assemblage. This suggests that three highly divergent species in terms of adaptive specializations, Alca, Alle, and Pinguinus, evolved from a single lineage in the Atlantic Ocean, in a process similar to the initial adaptive radiation of alcids in the Pacific Ocean.
An in silico search for P-transposable-element-related sequences in the Drosophila melanogaster genome allowed us to detect sequences that are similar to P-element transposases. These sequences are located in the central region of 3.4-kb Hoppel elements, a class II transposon. Polymerase chain reaction (PCR) analysis of the insertional polymorphism revealed that these elements are mobile. The 3.4-kb elements are the longest copies of this family ever found. They contain an open reading frame that is long enough to encode a transposase, suggesting that the 3.4-kb elements are the full-length copies of the Hoppel family. Multiple alignments of several P-element transposases from different species and the Hoppel-element-encoded peptide showed that all of the P-element introns and the 5’ region of the transposase are absent from the Hoppel sequence. Sequence analysis combined with reverse transcriptase PCR analysis showed that the 3.4-kb Hoppel elements are intronless. P and Hoppel not only share similar amino acid sequences but also have terminal inverted repeats of the same length (31 bp), and their excision footprints present a similar structure, which suggests that their transposases are functionally very similar. Thus, we propose that the Hoppel element family be included in the P-element superfamily. Two evolutionary scenarios are discussed considering the presence /absence of introns in the P-element superfamily.

The cosmopolitan genus Botrytis contains 22 recognized species and one hybrid. The current classification is largely based on morphological characters and, to a minor extent, on physiology and host range. In this study, a classification of the genus was constructed based on DNA sequence data of three nuclear protein-coding genes (RPB2, G3PDH, and HSP60) and compared with the traditional classification. Sexual reproduction and the host range, important fitness traits, were traced in the tree and used for the identification of major evolutionary events during speciation. The phylogenetic analysis corroborated the classical species delineation. In addition, the hybrid status of B. allii (B. byssoidea x B. aclada) was confirmed. Both individual gene trees and combined trees show that the genus Botrytis can be divided into two clades, radiating after the separation of Botrytis from other Sclerotiniaceae genera. Clade 1 contains four species that all colonize exclusively eudicot hosts, whereas clade 2 contains 18 species that are pathogenic on either eudicot (3) or monocot (15) hosts. A comparison of Botrytis and angiosperm phylogenies shows that cospeciation of pathogens and their hosts have not occurred during their respective evolution. Rather, we propose that host shifts have occurred during Botrytis speciation, possibly by the acquisition of novel pathogenicity factors. Loss of sexual reproduction has occurred at least three times and is supposed to be a consequence of negative selection.
Mecoptera and Siphonaptera represent two insect orders that have largely been overlooked in the study of insect vision. Recent phylogenetic evidence demonstrates that Mecoptera (scorpionflies) is paraphyletic, with the order Siphonaptera (fleas) nesting as sister to the family Boreidae (snow fleas), showing an evolutionary trend towards reduction in gross eye morphology within fleas. We provide the first molecular characterization of long-wavelength opsins from these three lineages (opsin gene from fleas [FL-Opsin], the Boreidae [B-Opsin], and a mecopteran family [M-Opsin]) and assess the effects of loss of visual acuity on the structure and function of the opsin gene. Phylogenetic analysis implies a physiological sensitivity in the red-green spectrum for these opsins. Analysis of intron splice sites reveals a high degree of similarity between FL-Opsin and B-Opsin as well as conserved splice sites across insect blue-green and long-wavelength opsins. Calculated rates of evolution and tests for destabilizing selection indicate that FL-Opsin, B-Opsin, and M-Opsin are evolving at similar rates with no radical selective pressures, implying conservative evolution and functional constraint across all three lineages.


http://mbe.oupjournals.org/cgi/content/abstract/21/1/29

Interleukin-13 (IL13) is believed to play an important role in the pathogenesis of atopy and allergic asthma. To better understand genetic variation at the IL13 locus, we resequenced a 5.1-kb genomic region spanning the entire locus and identified 26 single-nucleotide polymorphisms (SNPs) in 74 individuals from three major populations—Chinese, Caucasian, and African. Our survey suggests exceptionally high and significant geographic structure at the IL13 locus between African and outside Africa populations. This unusual pattern suggests that positive selection that acts in some local populations may have played a role on the IL13 locus. In support of this suggestion, we found a significant excess of high frequency-derived SNPs in the Chinese population and Caucasian population, respectively, as expected after a recent episode of positive selection. Further, the unusual haplotype structure indicates that different scenarios of the action of positive selection on the IL13 locus in different populations may exist. In the Caucasian population, the skewed haplotype distribution dominated by one common haplotype supports the hypothesis of simple directional selection. Whereas, in the Chinese population, the two-round hitchhiking hypothesis may explain the skewed haplotype structure with three dominant ones. These findings may provide insight into the likely relative roles of selection and population history in establishing present-day variation at the IL13 locus, and, motivate further studies of this locus as an important candidate in common diseases association studies.

Mol. Cancer Res. 2(2)


http://mcr.aacrjournals.org/cgi/content/abstract/1/5/385

Ligand-activated androgen receptors (ARs) occupy target genes and recruit histone modifiers that influence transcriptional competency. In LNCaP prostate cancer cells, the natural ligand 5(\alpha)\textsubscript{4}-dihydrotestosterone (DHT) activates transiently transfected AR-responsive promoter constructs;
concurrent treatment with the protein kinase A activator forskolin enhanced AR stimulation induced by DHT. Additional treatment with the cytokine IL-6, purportedly an AR activator, markedly inhibited receptor activity. To assess AR activity on natural chromatin-integrated promoters/enhancers, we determined AR occupancy of the endogenous prostate specific antigen (PSA) promoter/enhancer as well as PSA expression in LNCaP cells treated with DHT; AR occupancy of the PSA enhancer was rapid (within 1 h of stimulation), robust (10-fold over background), and sustained (8-16 h). In contrast, AR occupancy of the PSA promoter was only increased by 2-fold. Histone H3 acetylation at both the enhancer and promoter was evident 1-2 h after DHT treatment. Detectable pre- and mature PSA mRNA levels appeared after 1 and 6 h treatment, respectively. Substantial qualitative and quantitative differences in PSA expression and AR occupancy of the PSA enhancer were observed when DHT-induced and ligand-independent activations of the AR were compared; forskolin stimulated PSA mRNA and protein expression, whereas IL-6 inhibited both DHT- and forskolin-stimulated expression. IL-6 did not diminish DHT-dependent AR occupancy of the PSA enhancer but inhibited CBP/p300 recruitment, histone H3 acetylation, and cell proliferation. These findings provide a contextual framework for interpreting the contribution of non-steroidal activation of the AR to signaling in vivo, and have implications for prostate cancer cell growth.


http://mcr.aacrjournals.org/cgi/content/abstract/3/4/227

Emerging evidence shows that the stromal cell-derived factor 1 (SDF-1)/CXCR4 interaction regulates multiple cell signaling pathways and a variety of cellular functions such as cell migration, proliferation, and survival. There is little information linking the cellular functions and individual signaling pathways mediated by SDF-1 and CXCR4 in human cancer cells. In this study, we have shown that human epitheloid carcinoma HeLa cells express functional CXCR4 by reverse transcription-PCR, immunofluorescent staining, and 125I-SDF-1{alpha} ligand binding analyses. The treatment of HeLa cells with recombinant SDF-1{alpha} results in time-dependent Akt and extracellular signal-regulated kinase 1/2 (ERK1/2) activations. The SDF-1{alpha}-induced Akt and ERK1/2 activations are CXCR4 dependent as confirmed by their total inhibition by T134, a CXCR4-specific peptide antagonist. Cell signaling analysis with pathway-specific inhibitors reveals that SDF-1{alpha}-induced Akt activation is not required for ERK1/2 activation and vice versa, indicating that activations of Akt and ERK1/2 occur independently. Functional analysis shows that SDF-1{alpha} induces a CXCR4-dependent migration of HeLa cells. The migration can be totally blocked by phosphoinositide 3-kinase inhibitors, wortmannin or LY294002, whereas mitogen-activated protein/ERK kinase inhibitors, PD98059 and U0126, have no significant effect on SDF-1{alpha}-induced migration, suggesting that Akt activation, but not ERK1/2 activation, is required for SDF-1{alpha}-induced migration of epitheloid carcinoma cells.

Mol. Cancer Ther. (5)

XR5944 (MLN944) is a novel DNA targeting agent with potent antitumor activity, both in vitro and in vivo, against several murine and human tumor models. We have used an ATP-tumor chemosensitivity assay to assess the ex vivo sensitivity of a variety of solid tumors (n = 90) and a CCRF-CEM leukemia cell line selected with XR5944. Differences in gene expression between the parental CCRF-CEM and the resistant subline were investigated by quantitative reverse transcription-PCR. Immunohistochemistry for topoisomerases I and II(α) and multidrug resistance (MDR1) protein was done on those tumors for which tissue was available (n = 32). The CCRF-CEM XR5944 line showed increased mRNA levels of MDR1, major vault protein, and MDR-associated protein 1 compared with the parental line, whereas the expression of topoisomerases I, II(α), and II(β) was essentially unchanged, suggesting that XR5944 is susceptible to MDR mechanisms. The median IC90 and IC50 values for XR5944 in tumor-derived cells were 68 and 26 nmol/L, respectively, 6-fold greater than in resistant cell lines. XR5944 was 40- to 300-fold more potent than the other cytotoxics tested, such as doxorubicin, topotecan, and paclitaxel. Breast and gynecologic malignancies were most sensitive to XR5944, whereas gastrointestinal tumors showed greater resistance. A positive correlation (r = 0.68; P < 0.0001) was found between the IC50 values of XR5944 and P-glycoprotein/MDR1 staining but not with either topoisomerase I or II(α) immunohistochemistry index. These data support the rapid introduction of XR5944 to clinical trials and suggest that it may be effective against a broad spectrum of tumor types, especially ovarian and breast cancer.


The vitamin D3 receptor, which is the nuclear receptor for 1(α),25-dihydroxyvitamin D3 (VD3), forms a heterodimer with the retinoid X receptor (RXR), which is the nuclear receptor for 9-cis-retinoic acid (9-cis-RA). The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs, AGGTGA, spaced by three nucleotides [direct repeat (DR) 3] and modulates the expression of VD3-responsive genes. Telomerase activity, which is seen in most immortal cells and germ cells, is a complex of enzymes that maintain the length of telomeres. One of the major components of human telomerase, human telomerase reverse transcriptase (hTERT), is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity. We found that the sequence of 5'-AGTTTCATGGAGTTCA-3' (DR3') is similar to that of DR3 in the promoter region of hTERT. Our results showed that the combination of VD3 and 9-cis-RA inhibited telomerase activity through direct interaction of the heterodimer of vitamin D3 receptor and RXR with the DR3' sequence in the hTERT promoter as well as the combination of VD3 and selective RXR ligand did. Also, in vivo data showed that the growth of xenografts in nude mice was inhibited by VD3 and 9-cis-RA. The results of the present study provide evidence on the molecular mechanism of the inhibition of cell growth by these agents, and they could be novel therapeutic agents for prostate cancer.

Drug discovery strategies are needed that can rapidly exploit multiple therapeutic targets associated with the complex gene expression changes that characterize a polygenic disease such as cancer. We report a new cell-based high-throughput technology for screening chemical libraries against several potential cancer target genes in parallel. Multiplex gene expression (MGE) analysis provides direct and quantitative measurement of multiple endogenous mRNAs using a multiplexed detection system coupled to reverse transcription-PCR. A multiplex assay for six genes overexpressed in cancer cells was used to screen 9000 chemicals and known drugs in the human prostate cancer cell line PC-3. Active compounds that modulated gene expression levels were identified, and IC50 values were determined for compounds that bind DNA, cell surface receptors, and components of intracellular signaling pathways. A class of steroids related to the cardiac glycosides was identified that potently inhibited the plasma membrane Na+K+-ATPase resulting in the inhibition of four of the prostate target genes including transcription factors Hoxb-13, hPSE/PDEF, hepatocyte nuclear factor-3(α), and the inhibitor of apoptosis, survivin. Representative compounds selectively induced apoptosis in PC-3 cells compared with the nonmetastatic cell line BPH-1. The multiplex assay distinguished potencies among structural variants, enabling structure-activity analysis suitable for chemical optimization studies. A second multiplex assay for five toxicological markers, Hsp70, Gadd153, Gadd45, O6-methylguanine-DNA methyltransferase, and cyclophilin, detected compounds that caused DNA damage and cellular stress and was a more sensitive and specific indicator of potential toxicity than measurement of cell viability. MGE analysis facilitates rapid drug screening and compound optimization, the simultaneous measurement of toxicological end points, and gene function analysis.


http://mct.aacrjournals.org/cgi/content/abstract/1/3/215

The goal of this study was to determine the prevalence of sequence variants in the class I (β)-tubulin (clone m40) gene and their occurrence in human tumors and cancer cell lines. DNA was isolated from 93 control individuals representing a wide variety of ethnicities, 49 paclitaxel-naive specimens (16 ovarian cancers, 17 non-small cell lung cancers, and 16 ovarian cancer cell lines), and 30 paclitaxel-resistant specimens (9 ovarian cancers, 9 ovarian cancer cell lines, and 12 ovarian cancer xenografts in nude mice). Denaturing high-performance liquid chromatography and direct sequence analysis detected two silent polymorphisms in exon 4, Leu217Leu (CTG/CTA) and Gly400Gly (GGC/GGT), with minor allele frequencies of 17 and 0.5%, respectively. Five nucleotide substitutions and one single-base deletion were detected in introns 1, 2, and 3 and in the 3' untranslated region. Analysis of 49 paclitaxel-naive and 30 paclitaxel-resistant specimens revealed no additional polymorphisms in the coding region. In addition, no amino acid replacements were found in chimpanzee, gorilla, and orangutan in comparison to human. Our data demonstrate a very high degree of sequence conservation in class I (β)-tubulin, suggesting that all residues are important in tubulin structure and function. Individual variation in response to treatment with paclitaxel is not likely to be caused by genetic variations in the (β)-tubulin drug target. Moreover, acquired mutations in class I (β)-tubulin are unlikely to be a clinically relevant cause of drug resistance.


http://mct.aacrjournals.org/cgi/content/abstract/2/6/535
Previous studies have shown that decreased expression of the reduced folate carrier (RFC) and increased expression of dihydrofolate reductase (DHFR) are associated with intrinsic and acquired methotrexate resistance, respectively, in osteosarcoma (OS). It has also been shown in colorectal cancer that E2F-1 expression correlates with thymidylate synthase (TS) and, to a lesser extent, DHFR expression. To begin to investigate the regulation of DHFR and RFC expression in OS samples, mRNA expression of E2F-1 and E2F-4 were measured in OS tumor samples and related to DHFR, RFC, and TS mRNA expression. Using fluorescent quantitative real-time PCR, 112 human OS patient samples were investigated for potential E2F-1/E2F-4:DHFR, E2F-1/E2F-4:RFC, and E2F-1/E2F-4:TS correlations. The expression ranges for each gene are as follows: DHFR, 0.02-33.13 (median = 0.20); RFC, 0.02-229.13 (median = 1.91); TS, 0.01-9.99 (median = 0.15); E2F-1, 0.05-69.07 (median = 0.52); and E2F-4, 0.24-52.35 (median = 1.45). Spearman correlation coefficients (rs) for E2F-1:DHFR, E2F-1:RFC, E2F-1:TS, E2F-4:DHFR, E2F-4:RFC, and E2F-4:TS were calculated to be 0.53, 0.63, 0.60, 0.41, 0.58, and 0.33, respectively (P < 0.001). On the basis of this data, moderate correlations exist between E2F-1/E2F-4 and DHFR, RFC, and TS. These results suggest E2F-1/E2F-4 may play a role in the regulation of RFC expression, which has not been reported previously. The E2F transcription factors are also related to DHFR and TS expression in OS samples, suggesting a possible involvement in methotrexate resistance. Although E2F mRNA levels correlate with DHFR, RFC, and TS mRNA expression, additional experiments are necessary to determine the direct effects of these transcription factors and identify other proteins that may influence this relationship.

Mol. Cell. Biol. (37)


http://mcb.asm.org/cgi/content/abstract/25/6/2441

Vascular endothelial growth factor receptor 3 (Vegfr-3) is a tyrosine kinase that is expressed on the lymphatic endothelium and that signals for the growth of the lymphatic vessels (lymphangiogenesis). Vegf-d, a secreted glycoprotein, is one of two known activating ligands for Vegfr-3, the other being Vegf-c. Vegf-d stimulates lymphangiogenesis in tissues and tumors; however, its role in embryonic development was previously unknown. Here we report the generation and analysis of mutant mice deficient for Vegf-d. Vegf-d-deficient mice were healthy and fertile, had normal body mass, and displayed no pathologic changes consistent with a defect in lymphatic function. The lungs, sites of strong Vegf-d gene expression during embryogenesis in wild-type mice, were normal in Vegf-d-deficient mice with respect to tissue mass and morphology, except that the abundance of the lymphatics adjacent to bronchioles was slightly reduced. Dye uptake experiments indicated that large lymphatics under the skin were present in normal locations and were functional. Smaller dermal lymphatics were similar in number, location, and function to those in wild-type controls. The lack of a profound lymphatic phenotype in Vegf-d-deficient mice suggests that Vegf-d does not play a major role in lymphatic development or that Vegf-c or another, as-yet-unknown activating Vegfr-3 ligand can compensate for Vegf-d during development.

Bhattacharyya, S. N., S. Chatterjee, et al. (2002). "Mitochondrial RNA Import in Leishmania tropica: Aptamers Homologous to Multiple tRNA Domains That Interact Cooperatively or Antagonistically
A large number of cytoplasmic tRNAs are imported into the kinetoplast-mitochondrion of Leishmania by a receptor-mediated process. To identify the sequences recognized by import receptors, mitochondria were incubated with a combinatorial RNA library. Repeated cycles of amplification of the imported sequences (SELEX) resulted in rapid selection of several import aptamers containing sequence motifs present in the anticodon arm, the D arm, the V-T region, and acceptor stem of known tRNAs, confirming or suggesting the presence of import signals in these domains. As predicted, truncated derivatives of tRNA\textsubscript{Ile}(UAU) containing the D arm or the V-T region were imported in vitro. Four aptamers were studied in detail. All were imported in vitro as well as in transiently transfected cells, using the same pathway as tRNA, but their individual import efficiencies were different. Two types of aptamers were discernible: the A arm and D arm homologues (type I), which were efficiently transferred across the inner mitochondrial membrane, and the V-T homologues (type II), which were not. Remarkably, subnanomolar concentrations of type I RNAs stimulated the entry of type II RNAs into the matrix, whereas type II RNAs inhibited inner membrane transfer of type I RNAs. Moreover, tRNA\textsubscript{Tyr}(GUA) and tRNA\textsubscript{Ile}(UAU) interacted with one another as type I and type II, respectively. Such cooperative and antagonistic interactions may allow the use of a limited number of receptors to recognize a large number of tRNAs of variable affinity and enable the maintenance of a properly balanced tRNA pool for mitochondrial translation.
Neurospora crassa eliminates transgene-induced gene silencing (quelling) and the processing of dsRNA to an siRNA form. The two Dicer-like genes appear redundant because single mutants are quelling proficient. This first demonstration of the involvement of Dicer in gene silencing induced by transgenes supports a model by which a dsRNA produced by the activity of cellular RNA-dependent RNA polymerases on transgenic transcripts is an essential intermediate of silencing.


The SR family proteins and SR-related polypeptides are important regulators of pre-mRNA splicing. A novel SR-related protein of an apparent molecular mass of 53 kDa was isolated in a gene trap screen that identifies proteins which localize to the nuclear speckles. This novel protein possesses an arginine- and serine-rich domain and was termed SRrp53 (for SR-related protein of 53 kDa). In support for a role of this novel RS-containing protein in pre-mRNA splicing, we identified the mouse ortholog of the Saccharomyces cerevisiae U1 snRNP-specific protein Luc7p and the U2AF65-related factor HCC1 as interacting proteins. In addition, SRrp53 is able to interact with some members of the SR family of proteins and with U2AF35 in a yeast two-hybrid system and in cell extracts. We show that in HeLa nuclear extracts immunodepleted of SRrp53, the second step of pre-mRNA splicing is blocked, and recombinant SRrp53 is able to restore splicing activity. SRrp53 also regulates alternative splicing in a concentration-dependent manner. Taken together, these results suggest that SRrp53 is a novel SR-related protein that has a role both in constitutive and in alternative splicing.


Telomerase consists of two essential components, the telomerase RNA template (TR) and telomerase reverse transcriptase (TERT). The haplo-insufficiency of TR was recently shown to cause one form of human dyskeratosis congenita, an inherited disease marked by abnormal telomere shortening. Consistent with this finding, we recently reported that mice heterozygous for inactivation of mouse TR exhibit a similar haplo-insufficiency and are deficient in the ability to elongate telomeres in vivo. To further assess the genetic regulation of telomerase activity, we have compared the abilities of TR-deficient and TERT-deficient mice to maintain or elongate telomeres in interspecies crosses. Homozygous TERT knockout mice had no telomerase activity and failed to maintain telomere length. In contrast, TERT+/− heterozygotes had no detectable defect in telomere elongation compared to wild-type controls, whereas TR+/− heterozygotes were deficient in telomere elongation. Levels of TERT mRNA in heterozygous mice were one-third to one-half the levels expressed in wild-type mice, similar to the reductions in telomerase RNA observed in TR heterozygotes. These findings indicate that both TR and TERT are essential for telomere maintenance and elongation but that gene copy number and transcriptional regulation of TR, but not TERT, are limiting for telomerase activity under the in vivo conditions analyzed.

The ends of spontaneously occurring double-strand breaks (DSBs) may contain various lengths of single-stranded DNA, blocking lesions, and gaps and flaps generated by end annealing. To investigate the processing of such structures, we developed an assay in which annealed oligonucleotides are ligated onto the ends of a linearized plasmid which is then transformed into Saccharomyces cerevisiae. Reconstitution of a marker occurs only when the oligonucleotides are incorporated and repair is in frame, permitting rapid analysis of complex DSB ends. Here, we created DSBs with compatible overhangs of various lengths and asked which pathways are required for their precise repair. Three mechanisms of rejoining were observed, regardless of overhang polarity: nonhomologous end joining (NHEJ), a Rad52-dependent single-strand annealing-like pathway, and a third mechanism independent of the first two mechanisms. DSBs with overhangs of less than 4 bases were mainly repaired by NHEJ. Repair became less dependent on NHEJ when the overhangs were longer or had a higher GC content. Repair of overhangs greater than 8 nucleotides was as much as 150-fold more efficient, impaired 10-fold by rad52 mutation, and highly accurate. Reducing the microhomology extent between long overhangs reduced their repair dramatically, to less than NHEJ of comparable short overhangs. These data support a model in which annealing energy is a primary determinant of the rejoining efficiency and mechanism.


The activation of muscle-specific gene expression requires the coordinated action of muscle regulatory proteins and chromatin-remodeling enzymes. Microarray analysis performed in the presence or absence of a dominant-negative BRG1 ATPase demonstrated that approximately one-third of MyoD-induced genes were highly dependent on SWI/SNF enzymes. To understand the mechanism of activation, we performed chromatin immunoprecipitations analyzing the myogenin promoter. We found that H4 hyperacetylation preceded Brg1 binding in a MyoD-dependent manner but that MyoD binding occurred subsequent to H4 modification and Brg1 interaction. In the absence of functional SWI/SNF enzymes, muscle regulatory proteins did not bind to the myogenin promoter, thereby providing evidence for SWI/SNF-dependent activator binding. We observed that the homeodomain factor Pbx1, which cooperates with MyoD to stimulate myogenin expression, is constitutively bound to the myogenin promoter in a SWI/SNF-independent manner, suggesting a two-step mechanism in which MyoD initially interacts indirectly with the myogenin promoter and attracts chromatin-remodeling enzymes, which then facilitate direct binding by MyoD and other regulatory proteins.


WNK1 is a serine-threonine kinase, the expression of which is affected in pseudohypoaldosteronism type II, a Mendelian form of arterial hypertension. We characterized human WNK1 transcripts to determine the molecular mechanisms governing WNK1 expression.
We report the presence of two promoters generating two WNK1 isoforms with a complete kinase domain. Further variations are achieved by the use of two polyadenylation sites and tissue-specific splicing. We also determined the structure of a kidney-specific isoform regulated by a third promoter and starting at a novel exon. This transcript is kinase defective and has a predominant expression in the kidney compared to the other WNK1 isoforms, with, furthermore, a highly restricted expression profile in the distal convoluted tubule. We confirmed that the ubiquitous and kidney-specific promoters are functional in several cell lines and identified core promoters and regulatory elements. In particular, a strong enhancer element upstream from the kidney-specific exon seems specific to renal epithelial cells. Thus, control of human WNK1 gene expression of kinase-active or -deficient isoforms is mediated predominantly through the use of multiple transcription initiation sites and tissue-specific regulatory elements.


http://mcb.asm.org/cgi/content/abstract/24/12/5353

From the results of deletion analyses, the FERM domain of FAK has been proposed to inhibit enzymatic activity and repress FAK signaling. We have identified a sequence in the FERM domain that is important for FAK signaling in vivo. Point mutations in this sequence had little effect upon catalytic activity in vitro. However, the mutant exhibits reduced tyrosine phosphorylation and dramatically reduced Src family kinase binding. Further, the abilities of the mutant to transduce biochemical signals and to promote cell migration were severely impaired. The results implicate a FERM domain interaction in cell adhesion-dependent activation of FAK and downstream signaling. We also show that the purified FERM domain of FAK interacts with full-length FAK in vitro, and mutation of this sequence disrupts the interaction. These findings are discussed in the context of models of FAK regulation by its FERM domain.


http://mcb.asm.org/cgi/content/abstract/23/8/2749

Expression of the prosurvival Bcl-2 homologue Bfl-1/A1 is induced by NF-(kappa)B-activating stimuli, while B and T cells from c-rel knockout mice show an absolute defect in bfl-1/a1 gene activation. Here, we demonstrate NF-(kappa)B-dependent assembly of an enhanceosome-like complex on the promoter region of bfl-1. Binding of NF-(kappa)B subunit c-Rel to DNA nucleated the concerted binding of transcription factors AP-1 and C/EBP(beta) to the 5'-regulatory region of bfl-1. Optimal stability of the complex was dependent on proper orientation and phasing of the NF-(kappa)B site. Chromatin immunoprecipitation analyses demonstrated that T-cell activation triggers in vivo binding of endogenous c-Rel, c-Jun, C/EBP(beta), and HMG-IC to the bfl-1 regulatory region, coincident with selective recruitment of coactivators TAFII250 and p300, SWI/SNF chromatin remodeling factor component BRG-1, and basal transcription factors TATA-binding protein (TBP) and TFIIIB, as well as hyperacetylation of histones H3 and H4. These results highlight a critical role for NF-(kappa)B in bfl-1 transcription and point to the need for a complex and precise regulatory network to control bfl-1 expression. To our knowledge, this is the first demonstration of enhanceosome-mediated regulation of a cell death inhibitor.
Telomerase reintroduction in adult somatic tissues is envisioned as a way to extend their proliferative capacity. It is still a question, however, whether constitutive telomerase expression in adult tissues impacts the normal aging and spontaneous cancer incidence of an organism. Here, we studied the aging and spontaneous cancer incidence of mice with transgenic telomerase expression in a wide range of adult tissues, K5-Tert mice. For this, we maintained large colonies of K5-Tert mice for more than 2 years. K5-Tert mice showed a decreased life span compared to wild-type cohorts associated with a higher incidence of preneoplastic and neoplastic lesions in various tissue types. Neoplasias in K5-Tert mice were coincident with transgene expression in the affected tissues. These observations suggest that high telomerase activity may cooperate with genetic alterations that occur with age to promote tumorigenesis. Indeed, we demonstrate here that increased cancer incidence and the reduced viability of K5-Tert mice are aggravated in a p53+/- genetic background, indicating that telomerase cooperates with loss of p53 function in inducing tumorigenesis. Altogether, these results demonstrate that constitutive high levels of telomerase activity result in a decreased life span associated with an increased incidence of neoplasias as the organism ages.

Tumor necrosis factor alpha (TNF-α) and glucocorticoids are widely recognized as mutually antagonistic regulators of adaptive immunity and inflammation. Surprisingly, we show here that they cooperatively regulate components of innate immunity. The Toll-like receptor 2 (TLR2) gene encodes a transmembrane receptor critical for triggering innate immunity. Although TLR2 mRNA and protein are induced by inflammatory molecules such as TNF-α, we show that TLR2 is also induced by the anti-inflammatory glucocorticoids in cells where they also regulate MKP-1 mRNA and protein levels. TNF-α and glucocorticoids cooperate to regulate the TLR2 promoter, through the involvement of a 3′ NF-κB site, a STAT-binding element, and a 3′ glucocorticoid response element (GRE). Molecular studies show that the IκBα superrepressor or a STAT dominant negative element prevented TNF-α and dexamethasone stimulation of TLR2 promoter. Similarly, an AF-1 deletion mutant of glucocorticoid receptor or ablation of a putative GRE notably reduced the cooperative regulation of TLR2. Using chromatin immunoprecipitation assays, we demonstrate that all three transcription factors interact with both endogenous and transfected TLR2 promoters after stimulation by TNF-α and dexamethasone. Together, these studies define novel signaling mechanism for these three transcription factors, with a profound impact on discrimination of innate and adaptive immune responses.
DNA methylation is commonly associated with gene silencing, and a link between histone deacetylation and DNA methylation has been established. However, the transcriptional impact of the position and length of methylated zones relative to the promoter and the coding region of a gene remains quite unclear. This study investigates the impact of regional methylation on transcription and the relationship between DNA methylation and histone acetylation. Using patch-methylated stable episomes in human cells, we establish the pivotal importance of the location of DNA methylation in the regulation of transcription. We further demonstrate that the size of the methylated patch is not a key determinant for transcriptional suppression. The impact of DNA methylation on transcription is greater when it is in the transcription unit, and it is primarily a local effect. However, methylation outside of the transcription unit may potentiate the effect of methylation within the transcription unit. Acetylated histones are associated with unmethylated DNA and are nearly absent from methylated DNA regions. This association appears to be local and does not propagate along the DNA.


http://mcb.asm.org/cgi/content/abstract/23/12/4150

Establishment and maintenance of differential chromatin structure between transcriptionally competent and repressed genes are critical aspects of transcriptional regulation. The elements and mechanisms that mediate formation and maintenance of these chromatin states in vivo are not well understood. To examine the role of the promoter in maintaining chromatin structure and DNA methylation patterns of the transcriptionally active X-linked HPRT locus, 323 bp of the endogenous human HPRT promoter (from position -222 to +102 relative to the translation start site) was replaced by plasmid sequences by homologous recombination in cultured HT-1080 male fibrosarcoma cells. The targeted cells, which showed no detectable HPRT transcription, were then assayed for effects on DNase I hypersensitivity, general DNase I sensitivity, and DNA methylation patterns across the HPRT locus. In cells carrying the deletion, significantly diminished DNase I hypersensitivity in the 5' flanking region was observed compared to that in parental HT-1080 cells. However, general DNase I sensitivity and DNA methylation patterns were found to be very similar in the mutated cells and in the parental cells. These findings suggest that the promoter and active transcription play a relatively limited role in maintaining transcriptionally potentiated epigenetic states.


http://mcb.asm.org/cgi/content/abstract/23/18/6533

The human telomeric DNA binding factor TRF1 (hTRF1) and its interacting proteins TIN2, tankyrase 1 and 2, and P1NX1 have been implicated in the regulation of telomerase-dependent telomere length maintenance. Here we show that targeted deletion of exon 1 of the mouse gene encoding Trf1 causes early (day 5 to 6 postcoitus) embryonic lethality. The absence of telomerase did not alter the Terf1ex1{Delta}/ex1{Delta} lethality, indicating that the phenotype was not due to inappropriate telomere elongation by telomerase. Terf1ex1{Delta}/ex1{Delta} blastocysts had a severe growth defect of the inner cell mass that was accompanied by apoptosis. However, no evidence was found for telomere uncapping causing this cell death; chromosome spreads of Terf1ex1{Delta}/ex1{Delta} blastocysts did not reveal chromosome end-to-end fusions, and p53 deficiency only briefly delayed Terf1ex1{Delta}/ex1{Delta} lethality. These data suggest that murine Trf1 has an essential function that is independent of telomere length regulation.
Members of the homeobox family of transcription factors are major regulators of hematopoiesis. Overexpression of either HOXB4 or HOXA9 in primitive marrow cells enhances the expansion of hematopoietic stem cells (HSCs). However, little is known of how expression or function of these proteins is regulated during hematopoiesis under physiological conditions. In our previous studies we demonstrated that thrombopoietin (TPO) enhances levels of HOXB4 mRNA in primitive hematopoietic cells (K. Kirito, N. Fox, and K. Kaushansky, Blood 102:3172-3178, 2003). To extend our studies, we investigated the effects of TPO on HOXA9 in this same cell population. Although overall levels of the transcription factor were not affected, we found that TPO induced the nuclear import of HOXA9 both in UT-7/TPO cells and in primitive Sca-1+/c-kit+/Gr-1- hematopoietic cells in a mitogen-activated protein kinase-dependent fashion. TPO also controlled MEIS1 expression at mRNA levels, at least in part due to phosphatidylinositol 3-kinase activation. Collectively, TPO modulates the function of HOXA9 by leading to its nuclear translocation, likely mediated by effects on its partner protein MEIS1, and potentially due to two newly identified nuclear localization signals. Our data suggest that TPO controls HSC development through the regulation of multiple members of the Hox family of transcription factors through multiple mechanisms.
Phosphorylated derivatives of the lipid phosphatidylinositol are known to play critical roles in insulin response. Phosphatidylinositol 5-phosphate 4-kinases convert phosphatidylinositol 5-phosphate to phosphatidylinositol 4,5-bis-phosphate. To understand the physiological role of these kinases, we generated mice that do not express phosphatidylinositol 5-phosphate 4-kinase beta. These mice are hypersensitive to insulin and have reduced body weights compared to wild-type littermates. While adult male mice lacking phosphatidylinositol 5-phosphate 4-kinase beta have significantly less body fat than wild-type littermates, female mice lacking phosphatidylinositol 5-phosphate 4-kinase (beta) have increased insulin sensitivity in the presence of normal adiposity. Furthermore, in vivo insulin-induced activation of the protein kinase Akt is enhanced in skeletal muscle and liver from mice lacking phosphatidylinositol 5-phosphate 4-kinase (beta). These results indicate that phosphatidylinositol 5-phosphate 4-kinase (beta) plays a role in determining insulin sensitivity and adiposity in vivo and suggest that inhibitors of this enzyme may be useful in the treatment of type 2 diabetes.


The ubiquitously expressed E4F protein was originally identified as an E1A-regulated cellular transcription factor required for adenovirus replication. The function of this protein in normal cell physiology remains largely unknown. To address this issue, we generated E4F knockout mice by gene targeting. Embryos lacking E4F die at the peri-implantation stage, while in vitro-cultured E4F/- blastocysts exhibit defects in mitotic progression, chromosomal missegregation, and...
increased apoptosis. Consistent with these observations, we found that E4F localizes to the mitotic spindle during the M phase of early embryos. Our results establish a crucial role for E4F during early embryonic cell cycles and reveal an unexpected function for E4F in mitosis.


http://mcbr.asm.org/cgi/content/abstract/22/23/8199

To examine the physiological functions of mannose-binding lectin A (MBL-A), we generated mice that were deficient in MBL-A and examined their susceptibilities to the microbial pathogens Candida albicans and Plasmodium yoelii, an accepted experimental malaria model in mouse. We found no differences in the survival rates and fungal burdens of wild-type and MBL-A-/- mice with disseminated C. albicans infection. The two mouse strains were also similar in their abilities to resist hepatic accumulation of P. yoelii parasites. We conclude that MBL-A deficiency does not alter resistance to disseminated candidiasis or initial hepatic invasion by P. yoelii.


http://mcbr.asm.org/cgi/content/abstract/23/6/1946

Methylation of cytosine in CpG dinucleotides promotes transcriptional repression in mammals by blocking transcription factor binding and recruiting methyl-binding proteins that initiate chromatin remodeling. Here, we use a novel cell-based system to show that retrovirally expressed Pax-5 protein activates endogenous early B-cell-specific mb-1 genes in plasmacytoma cells, but only when the promoter is hypomethylated. CpG methylation does not directly affect binding of the promoter by Pax-5. Instead, methylation of an adjacent CpG interferes with assembly of ternary complexes comprising Pax-5 and Ets proteins. In electrophoretic mobility shift assays, recruitment of Ets-1 is blocked by methylation of the Ets site (5'CCGGAG) on the antisense strand. In transfection assays, selective methylation of a single CpG within the Pax-5-dependent Ets site greatly reduces mb-1 promoter activity. Prior demethylation of the endogenous mb-1 promoter is required for its activation by Pax-5 in transduced cells. Although B-lineage cells have only unmethylated mb-1 genes and do not modulate methylation of the mb-1 promoter during development, other tissues feature high percentages of methylated alleles. Together, these studies demonstrate a novel DNA methylation-dependent mechanism for regulating transcriptional activity through the inhibition of DNA-dependent protein-protein interactions.


http://mcbr.asm.org/cgi/content/abstract/22/8/2598

The transcriptional coactivator p300 regulates transcription by binding to proteins involved in transcription and by acetylating histones and other proteins. These transcriptional effects are mainly at promoter and enhancer elements. Regulation of transcription also occurs through scaffold/matrix attachment regions (S/MARs), the chromatin regions that bind the nuclear matrix. Here we show that p300 binds to the S/MAR binding protein scaffold attachment factor A (SAF-
A), a major constituent of the nuclear matrix. Using chromatin immunoprecipitations, we established that both p300 and SAF-A bind to S/MAR elements in the transiently silent topoisomerase I gene prior to its activation at G1 during cell cycle. This binding is accompanied by local acetylation of nucleosomes, suggesting that p300-SAF-A interactions at S/MAR elements of nontranscribed genes might poise these genes for transcription.


Gene activation in eukaryotes requires chromatin remodeling, in part via histone modifications. To study the events at the promoter of a mitogen-inducible gene, we examined the induction of expression of the collagenase gene. It has been established that the collagenase gene can be activated by c-Jun and c-Fos and that the transcriptional coactivator p300 is involved in the activation. As expected, we found histone acetyltransferase activity at the collagenase promoter during activation. Interestingly, we also found histone methyltransferase and kinase activity. Strikingly, the first modification observed is methylation of histone H3 lysine 4, which correlates with the binding of the SET9 methyltransferase and the assembly of a complex consisting of c-Jun, c-Fos, TATA binding protein, and RNA polymerase II. The assembly of the preinitiation complex also shows an ordered binding of the acetyltransferase p300, the RSK2 kinase, and the SWI/SNF component Brg-1. Our results suggest that collagenase gene activation involves a dynamic recruitment of different factors and that in addition to acetylation, histone H3 lysine 4 di- and trimethylation and histone H3 serine 10 phosphorylation are important steps in the activation of this gene.


Differentiating male germ cells express a testis-specific form of cytochrome c (Cyt cT) that is distinct from the cytochrome c expressed in somatic cells (Cyt cS). To examine the role of Cyt cT in germ cells, we generated mice null for Cyt cT. Homozygous Cyt cT-/- pups were statistically underrepresented (21%) but developed normally and were fertile. However, spermatozoa isolated from the cauda epididymis of Cyt cT-null animals were less effective in fertilizing oocytes in vitro and contain reduced levels of ATP compared to wild-type sperm. Sperm from Cyt cT-null mice contained a greater number of immotile spermatozoa than did samples from control mice, i.e., 53.1% {+/-} 13.7% versus 33.2% {+/-} 10.3% (P < 0.0001) for vas deferens sperm and 40.1% {+/-} 9.6% versus 33.2% {+/-} 7.5% (P = 0.0104) for epididymal sperm. Cyt cT-null mice often exhibit early atrophy of the testes after 4 months of age, losing germ cells as a result of increased apoptosis. However, no difference in the activation of caspase-3, -8, or -9 was detected between the Cyt cT-/- testes and controls. Our data indicate that the Cyt cT-null testes undergo early atrophy equivalent to that which occurs during aging as a consequence of a reduction in oxidative phosphorylation.

Low-molecular-weight protein tyrosine phosphatase (LMW-PTP) has been implicated in the regulation of cell growth and actin rearrangement mediated by several receptor tyrosine kinases, including platelet-derived growth factor and epidermal growth factor. Here we identify the Xenopus laevis homolog of LMW-PTP1 (XLPTP1) as an additional positive regulator in the fibroblast growth factor (FGF) signaling pathway during Xenopus development. XLPTP1 has an expression pattern that displays substantial overlap with FGF receptor 1 (FGFR1) during Xenopus development. Using morpholino antisense technology, we show that inhibition of endogenous XLPTP1 expression dramatically restricts anterior and posterior structure development and inhibits mesoderm formation. In ectodermal explants, loss of XLPTP1 expression dramatically blocks the induction of the early mesoderm gene, Xbrachyury (Xbra), by FGF and partially blocks Xbra induction by Activin. Moreover, FGF-induced activation of mitogen-activated protein (MAP) kinase is also inhibited by XLPTP1 morpholino oligonucleotides; however, introduction of RNA encoding XLPTP1 is able to rescue morphological and biochemical effects of antisense inhibition. Inhibition of FGF-induced MAP kinase activity due to loss of XLPTP1 is also rescued by an active Ras, implying that XLPTP1 may act upstream of or parallel to Ras. Finally, XLPTP1 physically associates only with an activated FGFR1, and this interaction requires the presence of SNT1/FRS-2 (FGFR substrate 2). Although LMW-PTP1 has been shown to participate in other receptor systems, the data presented here also reveal XLPTP1 as a new and important component of the FGF signaling pathway.


Chromatin structure is believed to exert a strong effect on replication origin function. We have studied the replication of the chicken {beta}-globin locus, whose chromatin structure has been extensively characterized. This locus is delimited by hypersensitive sites (HSSs) that mark the position of insulator elements. A stretch of condensed chromatin and another HS separate the {beta}-globin domain from an adjacent folate receptor (FR) gene. We demonstrate here that in erythroid cells that express the FR but not the globin genes, replication initiates at four sites within the {beta}-globin domain, one at the 5' HS4 insulator and the other three near the (rho)- and (beta)A-globin genes. Three origins consist of G+C-rich sequences enriched in CpG dinucleotides. The fourth origin is A+T rich. Together with previous work, these data reveal that the insulator origin has unmethylated CpGs, hyperacetylated histones H3 and H4, and lysine 4-methylated histone H3. In contrast, opposite modifications are observed at the other G+C-rich origins. We also show that the whole region, including the stretch of condensed chromatin, replicates early in S phase in these cells. Therefore, different early-firing origins within the same locus may have opposite patterns of epigenetic modifications. The role of insulator elements in DNA replication is discussed.

analyzed the consequences of disrupting the CENP-A gene in the chicken DT40 cell line. In CENP-A-depleted cells, kinetochore protein assembly is impaired, as indicated by mislocalization of the inner kinetochore proteins CENP-I, CENP-H, and CENP-C as well as the outer components Nuf2/Hec1, Mad2, and CENP-E. However, BubR1 and the inner centromere protein INCENP are efficiently recruited to kinetochores. Following CENP-A depletion, chromosomes are deficient in proper congression on the mitotic spindle and there is a transient delay in prometaphase. CENP-A-depleted cells further proceed through anaphase and cytokinesis with unequal chromosome segregation, suggesting that some kinetochore function remains following substantial depletion of CENP-A. We furthermore demonstrate that CENP-A-depleted cells exhibit a specific defect in maintaining kinetochore localization of the checkpoint protein BubR1 under conditions of checkpoint activation. Our data thus point to a specific role for CENP-A in assembly of kinetochores competent in the maintenance of mitotic checkpoint signaling.


http://mcb.asm.org/cgi/content/abstract/24/5/1855

We have examined the alternative splicing of the Drosophila melanogaster prospero twintron, which contains splice sites for both the U2- and U12-type spliceosome and generates two forms of mRNA, pros-L (U2-type product) and pros-S (U12-type product). We find that twintron splicing is developmentally regulated: pros-L is abundant in early embryogenesis while pros-S displays the opposite pattern. We have established a Kc cell in vitro splicing system that accurately splices a minimal pros substrate containing the twintron and have examined the sequence requirements for pros twintron splicing. Systematic deletion and mutation analysis of intron sequences established that twintron splicing requires a 46-nucleotide purine-rich element located 32 nucleotides downstream of the U2-type 5' splice site. While this element regulates both splicing pathways, its alteration showed the severest effects on the U2-type splicing pathway. Addition of an RNA competitor containing the wild-type purine-rich element to the Kc extract abolished U2-type splicing and slightly repressed U12-type splicing, suggesting that a trans-acting factor(s) binds the enhancer element to stimulate twintron splicing. Thus, we have identified an intron region critical for prospero twintron splicing as a first step towards elucidating the molecular mechanism of splicing regulation involving competition between the two kinds of spliceosomes.


http://mcb.asm.org/cgi/content/abstract/24/14/6403

TACC2 is a member of the transforming acidic coiled-coil-containing protein family and is associated with the centrosome-spindle apparatus during cell cycling. In vivo, the TACC2 gene is expressed in various splice forms predominantly in postmitotic tissues, including heart, muscle, kidney, and brain. Studies of human breast cancer samples and cell lines suggest a putative role of TACC2 as a tumor suppressor protein. To analyze the physiological role of TACC2, we generated mice lacking TACC2. TACC2-deficient mice are viable, develop normally, are fertile, and lack phenotypic changes compared to wild-type mice. Furthermore, TACC2 deficiency does not lead to an increased incidence of tumor development. Finally, in TACC2-deficient embryonic fibroblasts, proliferation and cell cycle progression as well as centrosome numbers are comparable to those in wild-type cells. Therefore, TACC2 is not required, nonredundantly, for mouse development and normal cell proliferation and is not a tumor suppressor protein.
The transcription factor E2A can promote precursor B cell expansion, promote G1 cell cycle progression, and induce the expressions of multiple G1-phase cyclins. To better understand the mechanism by which E2A induces these cyclins, we characterized the relationship between E2A and the cyclin D3 gene promoter. E2A transactivated the 1-kb promoter of cyclin D3, which contains two E boxes. However, deletion of the E boxes did not disrupt the transactivation by E2A, raising the possibility of indirect activation via another transcription factor or binding of E2A to non-E-box DNA elements. To distinguish between these two possibilities, promoter occupancy was examined using the DamID approach. A fusion construct composed of E2A and the Escherichia coli DNA adenosine methyltransferase (E47Dam) was subcloned in lentivirus vectors and used to transduce precursor B-cell and myeloid progenitor cell lines. In both cell types, specific adenosine methylation was identified at the cyclin D3 promoter. Chromatin immunoprecipitation analysis confirmed the DamID findings and localized the binding to within 1 kb of the two E boxes. The methylation by E47Dam was not disrupted by mutations in the E2A portion that block DNA binding. We conclude that E2A can be recruited to the cyclin D3 promoter independently of E boxes or E2A DNA binding activity.

S100B is a Ca2+-modulated protein of the EF-hand type with both intracellular and extracellular roles. S100B, which is most abundant in the brain, has been shown to exert trophic and toxic effects on neurons depending on the concentration attained in the extracellular space. S100B is also found in normal serum, and its serum concentration increases in several nervous and nonnervous pathological conditions, suggesting that S100B-expressing cells outside the brain might release the protein and S100B might exert effects on nonnervous cells. We show here that at picomolar to nanomolar levels, S100B inhibits myogenic differentiation of rat L6 myoblasts via inactivation of p38 kinase with resulting decrease in the expression of the myogenic differentiation markers, myogenin, muscle creatine kinase, and myosin heavy chain, and reduction of myotube formation. Although myoblasts express the multiligand receptor RAGE, which has been shown to transduce S100B effects on neurons, S100B produces identical effects on myoblasts overexpressing either full-length RAGE or RAGE lacking the transducing domain. This suggests that S100B affects myoblasts by interacting with another receptor and that RAGE is not the only receptor for S100B. Our data suggest that S100B might participate in the regulation of muscle development and regeneration by inhibiting crucial steps of the myogenic program in a RAGE-independent manner.
{zeta} that is implicated in mutagenic translesion synthesis of damaged DNA. To investigate the function of its mouse homologue, we have generated mouse embryonic stem cells and mice carrying a targeted disruption of Rev3. Although some strain-dependent variation was observed, Rev3-/− embryos died around midgestation, displaying retarded growth in the absence of consistent developmental abnormalities. Rev3-/− cell lines could not be established, indicating a cell-autonomous requirement of Rev3 for long-term viability. Histochemical analysis of Rev3-/− embryos did not reveal aberrant replication or cellular proliferation but demonstrated massive apoptosis in all embryonic lineages. Although increased levels of p53 are detected in Rev3-/− embryos, the embryonic phenotype was not rescued by the absence of p53. A significant increase in double-stranded DNA breaks as well as chromatid and chromosome aberrations was observed in cells from Rev3-/− embryos. The inner cell mass of cultured Rev3-/− blastocysts dies of a delayed apoptotic response after exposure to a low dose of N-acetoxy-2-acetylaminofluorene. These combined data are compatible with a model in which, in the absence of polymerase {zeta}, double-stranded DNA breaks accumulate at sites of unreplicated DNA damage, eliciting a p53-independent apoptotic response. Together, these data are consistent with involvement of polymerase {zeta} in translesion synthesis of endogenously and exogenously induced DNA lesions.


http://mcb.asm.org/cgi/content/abstract/23/19/7055

Posttranscriptional controls in higher eukaryotes are central to cell differentiation and developmental programs. These controls reflect sequence-specific interactions of mRNAs with one or more RNA binding proteins. The {alpha}-globin poly(C) binding proteins (αCPs) comprise a highly abundant subset of KH domain RNA binding proteins and have a characteristic preference for binding single-stranded C-rich motifs. αCPs have been implicated in translation control and stabilization of multiple cellular and viral mRNAs. To explore the full contribution of αCPs to cell function, we have identified a set of mRNAs that associate in vivo with the major αCP2 isoforms. One hundred sixty mRNA species were consistently identified in three independent analyses of αCP2-RNP complexes immunopurified from a human hematopoietic cell line (K562). These mRNAs could be grouped into subsets encoding cytoskeletal components, transcription factors, proto-oncogenes, and cell signaling factors. Two mRNAs were linked to ceroid lipofuscinosis, indicating a potential role for αCP2 in this infantile neurodegenerative disease. Surprisingly, αCP2 mRNA itself was represented in αCP2-RNP complexes, suggesting autoregulatory control of αCP2 expression. In vitro analyses of representative target mRNAs confirmed direct binding of αCP2 within their 3′ untranslated regions. These data expand the list of mRNAs that associate with αCP2 in vivo and establish a foundation for modeling its role in coordinating pathways of posttranscriptional gene regulation.


http://mcb.asm.org/cgi/content/abstract/23/11/3837

Early B-cell factor (EBF) is a DNA binding protein required for early B-cell development. It activates transcription of several B-cell-specific genes, including the λ5 gene, which encodes a protein necessary for signaling by the pre-B-cell receptor. In an effort to understand the mechanism by which EBF activates transcription, we examined its interaction with the coactivator protein p300/CBP. We found that two domains of EBF each bind the histone
acetyltransferase (HAT)/CH3 domain of p300/CBP both in vitro and in vivo. Surprisingly, transcriptional activation by EBF was not sensitive to E1A, a potent p300/CBP inhibitor. In fact, overexpressed EBF mimicked E1A by severely repressing the activity of several other transcription factors, including E47, a protein that acts cooperatively with EBF to promote transcription of the \( \lambda5 \) gene. This broad inhibitory profile correlated with EBF's ability to repress the HAT activity of p300/CBP in vivo and in vitro. However, such a repressed complex is not likely to form at the \( \lambda5 \) promoter in vivo since (i) EBF could not bind p300/CBP and DNA simultaneously and (ii) the cooperativity imparted by E47 was sensitive to E1A. Our data reveal an intriguing inhibitory property of EBF—a property shared only by E1A, Twist, Pu.1, and the Hox family of homeodomain proteins—and suggest that E47 and EBF play distinct roles during \( \lambda5 \) promoter activation.


http://mcb.asm.org/cgi/content/abstract/24/22/10047

A minimal amount of extranucleosomal DNA was required for nucleosome mobilization by ISW2 as shown by using a photochemical histone mapping approach to analyze nucleosome movement on a set of nucleosomes with varied lengths of extranucleosomal DNA. ISW2 was ineffective in repositioning or mobilizing nucleosomes with \[ \leq \] 20 bp of extranucleosomal DNA. In addition, ISW2 was able to slide nucleosomes to within only 10 to 13 bp of the edge of DNA fragments. The nucleosome mobilization was promoted by extranucleosomal single-stranded DNA with modest strand preference. Gaps (10 bp) just inside the nucleosome and in the extranucleosomal DNA showed that the transfer of torsional strain (twist) into the nucleosomal DNA region was not required for mobilizing nucleosomes. However, indications are that the extranucleosomal DNA immediately adjacent to the nucleosome has an important role in the initial stage of nucleosome movement by ISW2.


http://jmd.amjpathol.org/cgi/content/abstract/7/2/283

We report nine novel DNA alterations in the RET proto-oncogene in 12 unrelated cases identified by DNA sequencing of exons 10 and 11 of the gene. The novel variants K666E, IVS9-11G[&lt;gt]A, D631V in cis with H665Q, D631E (with C634Y), E623K (in trans with C618S), 616delGAG (in trans with C609Y), Y606C, C630R, and R635-T636insELCR;T636P were detected in patients with various clinical presentations ranging from thyroid goiter, medullary thyroid carcinoma, and pheochromocytoma to classic multiple endocrine neoplasia type 2A. When novel DNA alterations are found, extended family studies can be helpful in determining the clinical significance of such findings. Segregation within families suggests that K666E and T636insELCR;T636P are likely to be disease-causing mutations. However, the mechanism by which they affect the normal activity of the RET receptor is unclear. Absence of segregation with disease was observed for E623K and 616delGAG. For the remainder of the DNA alterations, family studies were not possible, and
the clinical significance of these novel variants needs further assessment. Additional case reports, animal models, and/or functional studies are needed to determine the clinical significance of these newly identified variants.


http://jmd.amjpathol.org/cgi/content/abstract/4/3/159

Helicobacter pylori (HP) causes dense gastritis that can be difficult to distinguish morphologically from MALT-type lymphoma (ML). Immunoglobulin heavy chain (IgH) gene analysis by polymerase chain reaction (PCR) is often used to resolve diagnosis. However, monoclonal bands have been reported in nonmalignant cases of gastritis. Retrospectively, 16 gastric ML with both formalin-fixed, paraffin-embedded (FF-PE) and ethanol-fixed samples (EF), and 9 cases of FF-PE HP-gastritis were analyzed by IgH PCR to document the presence of non-reproducible bands in HP-gastritis, but not ML samples. In duplicate analyses, 12 of 16 ML yielded identical monoclonal bands in FF-PE and EF samples whereas 3 of 9 FF-PE gastritis cases yielded different-sized (ie, non-reproducible) "clonal" bands. Sequencing of two PCR products from a gastritis case confirmed IgH gene sequences. To investigate whether FF-PE had a direct effect on producing these non-reproducible bands, 7 gastrectomy samples were prospectively divided into EF and FF-PE portions while 4 of 7 FF-PE portions yielded either multiple distinct bands or non-reproducible bands. In conclusion, IgH PCR of FF-PE tissue can create artifactual "clonal" bands, which are the appropriate product size, contain IgH sequences, and, if not performed in duplicate, may confuse interpretation of B-cell clonality.


http://jmd.amjpathol.org/cgi/content/abstract/4/1/44

Clear cell sarcoma (CCS), also known as melanoma of soft parts, is an uncommon deep soft tissue tumor presenting typically in the lower extremities of young adults. Previous cytogenetic studies have established the specificity of the recurrent t(12;22)(q13;q12), resulting in a EWS-ATF1 fusion, for CCS. The prevalence of the EWS-ATF1 fusion in CCS remains unclear, since most genetically confirmed CCS have been reported as isolated cytogenetic or molecular diagnostic case reports. We therefore studied histologically confirmed CCS from 12 patients for the presence of EWS-ATF1 by reverse-transcriptase polymerase chain reaction (RT-PCR), using RNA extracted from either frozen (four cases) or formalin-fixed paraffin-embedded (eight cases) material. All primary tumors were located in the deep soft tissues of the extremities. Histologically, 10 cases had a typical epithelioid nested appearance. Most or all cases showed immunostaining for HMB45 (12 of 12), S-100 protein (10 of 12), and MITF (12 of 12). Ultrastructural analysis showed melanosomes in six of seven cases. The presence of an EWS-ATF1 fusion transcript was identified by RT-PCR in 11 of 12 cases (91%), all of which showed the same fusion transcript structure, namely the previously described in-frame fusion of EWS exon 8 to ATF1 codon 65. RT-PCR analysis for the melanocyte-specific splice form of the MITF transcript was positive in all cases tested (4 of 4). These data confirm that EWS-ATF1 detection can be used as a highly sensitive diagnostic test for CCS and that CCS expresses the melanocyte-specific form of the MITF transcript, further supporting its genuine melanocytic differentiation.

http://jmd.amjpathol.org/cgi/content/abstract/5/3/168

Fluorescence in situ hybridization (FISH) has been used to demonstrate the t(14;18) in up to 100% of follicular lymphoma (FL) cases, however, there is little reproducible data using fixed tissue. The aim was therefore to develop a robust FISH method for the demonstration of translocations in archival tissue. The technique was evaluated by comparison with multiplex polymerase chain reaction (PCR), capable of detecting the majority of known breakpoints. Twenty-eight paired frozen and fixed cases of FL and 20 reactive controls were analyzed. The t(14;18) was detected in 23 of 28 cases using PCR on frozen material and 8 of 20 in paraffin. Using FISH, 24 of 26 frozen and 26 of 28 paraffin cases had a demonstrable translocation. All 20 reactive nodes were negative for the t(14;18) by PCR. Using FISH, one of the reactive cases had occasional cells with a translocation FISH pattern, demonstrable in frozen and paraffin samples. This is consistent with the presence of the t(14;18), which is well described in normal individuals. Both PCR and FISH are highly effective for t(14;18) analysis in unfixed tissue. When only paraffin blocks are available, FISH is the method of choice, and a result was achieved in 100% of cases. The method is applicable to the retrospective analysis of a range of translocations.


http://jmd.amjpathol.org/cgi/content/abstract/7/2/226

A significant fraction of hereditary nonpolyposis colorectal cancer cases with defective mismatch repair (ie, Lynch syndrome) have large genomic deletions or duplications in the mismatch repair genes, hMLH1 and hMSH2, which can be challenging to detect by traditional methods. For this study, we developed and validated a novel Southern blot analysis method that allows for ascertainment of the extent of the dosage alterations on an exon-by-exon basis and compared this method to a second novel technique, multiplex ligation-dependent probe amplification (MLPA). From a total of 254 patients referred for Lynch syndrome testing, 20 of the 118 MLH1 cases and 42 of the 136 MSH2 cases had large genomic alterations, as detected by Southern blot. MLPA and Southern blot results were concordant with the exception of three major discrepancies: one because of a lack of MLPA probes for the region altered, another because of a point mutation near the MLPA probe ligation site, and another that was unexplained. Compared to Southern blot, MLPA has a shorter turn-around time, the analysis is less costly, less time-consuming, and less labor-intensive, and results are generally clear and unambiguous. However, concerns with MLPA include the presence of false-negatives and -positives because of positioning of probes and DNA variants near the probe ligation site. Overall, both Southern blot and MLPA provide important tools for the complete evaluation of patients with Lynch syndrome.


http://jmd.amjpathol.org/cgi/content/abstract/7/1/17
Kaposi's sarcoma-associated herpesvirus (KSHV), also termed human herpesvirus type 8, is consistently identified in Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease. Here we report four cases of KSHV-bearing solid lymphomas that occurred in AIDS patients (cases 1 to 3) and in a human immunodeficiency virus (HIV)-seronegative person (case 4). The patients presented extranodal masses in the abdomen (cases 1, 3, and 4) or skin (case 2), and nodal involvement, together with Kaposi's sarcoma (case 3). The gastrointestinal tract was involved in two patients (cases 1 and 3). The patients did not develop a lymphomatous effusion. KSHV was detected in the tumor cells of all cases by immunohistochemistry and by polymerase chain reaction. Epstein-Barr virus was detected in two of the HIV-related cases. All KSHV-positive solid lymphomas exhibited PEL-like cell morphology. To investigate the relationship of these disorders to PEL and to other AIDS-associated diffuse large cell lymphomas, KSHV-positive solid lymphomas were tested for the expression of a set of genes that were previously shown by gene profiling analysis to define PEL tumor cells. The results showed that expression of this set of genes in KSHV-positive lymphomas is similar to that of PEL but distinct from KSHV-negative AIDS-associated diffuse large cell lymphomas. Because pathobiological features of KSHV-positive solid lymphomas closely mimic those of PEL, our results suggest that KSHV-positive solid lymphomas should be considered as a tissue-based variant of classical PEL, irrespective of HIV status.


http://jmd.amjpathol.org/cgi/content/abstract/4/2/108

The autosomal-dominant spinocerebellar ataxias (ADCA) are a heterogeneous group of neurodegenerative disorders with variable expression and phenotypic overlap. An accurate diagnosis relies on detection of a mutation in a specific causative gene, which is typically an abnormal number of CAG trinucleotide repeats. To streamline testing in a clinical setting, we converted our current panel of tests for the spinocerebellar ataxias (SCA) types SCA1, SCA2, SCA3, SCA6, and SCA7 from five independent amplification reactions analyzed by polyacrylamide gel electrophoresis (PAGE) to a single multiplex amplification reaction analyzed by capillary electrophoresis (CE). Multiplex amplification was facilitated by the use of chimeric primers; different lengths and fluorochromes distinguished the amplicons. During CE with commercially available molecular weight standards, the SCA amplicons migrated faster than predicted, thereby underestimating their length compared to that determined previously by PAGE. This was observed to varying degrees for each of the five loci, with the greatest size differential occurring in amplicons with greater (CAG)n. To determine accurate amplicon length, and therefore an accurate number of CAG repeats, a size correction formula was calculated for each locus. This multiplex semi-automated assay has been reliable during 1 year of use in a clinical setting during which 57 samples were tested and five positive samples were detected.


http://jmd.amjpathol.org/cgi/content/abstract/6/4/348

The development of simple and rapid methods for the detection of the common genetic mutations associated with cystic fibrosis (CF) requires access to positive-control samples including the 5/7/9T variants of intron 8. We used PCR and a simple multiplex bead-array assay to identify 5/7/9T control samples from 29 commercially available DNA samples. Unpurified PCR products were directly hybridized to color-coded beads containing allele-specific capture probes for 5/7/9T
detection. The performance of the assay was investigated using reverse-complement oligonucleotides, individual PCR products, and multiplex PCR products for 5/7/9T detection within a complex CFTR screening assay. Samples were genotyped by grouping the relative signal intensities from each capture probe. Of 29 commercially available DNA samples analyzed, 2 5T/7T, 2 5T/9T, 9 7T/9T, 11 7T/7T, and 5 9T/9T genotypes were identified. The genotype within each sample group was confirmed by DNA sequencing. The assay was compatible with the analysis of 10 to 1000 ng of genomic DNA isolated from whole blood and allowed for the separate identification of primary CFTR mutations from reflex variants. The correct identification of positive controls demonstrated the utility of a simple bead-array assay and provided accessible samples for assay optimization and for routine quality control in the clinical laboratory.


http://jmd.amjpathol.org/cgi/content/abstract/4/2/103

Polymerase chain reaction with confronting two-pair primers (PCR-CTPP) is an inexpensive, time-saving genotyping method that is applicable for most single nucleotide polymorphisms. To date, we have applied PCR-CTPP successfully for the genotyping of more than 30 polymorphisms. This paper demonstrates the differences in DNA amplification among different annealing temperatures of PCR-CTPP with given melting temperatures for four primers. The NQO1 C609T (Pro187Ser) polymorphism was used as an example. Two sets of four primers were applied for PCR-CTPP; the first set with different melting temperatures (Tms), and the second with similar Tms. The comparisons with one-pair primer PCR (allele-specific PCR) revealed that PCR-CTPP amplified DNA more specifically than allele-specific PCR. The primers with different Tms caused competitive DNA amplification for heterozygous genotype. Four primers with similar Tms amplified both alleles unspecifically at a lower annealing temperature, while the same DNA samples were correctly genotyped under an optimal annealing temperature. These findings are unique for PCR-CTPP, and important characteristics when the primers and annealing temperatures in PCR-CTPP are designed. The knowledge of these characteristics will extend the applicability of PCR-CTPP for polymorphism genotyping.


http://jmd.amjpathol.org/cgi/content/abstract/5/1/15

Human Epstein-Barr virus (EBV) and cytomegalovirus (CMV) can cause serious complications in immunocompromised patients. Rapid diagnosis of EBV and CMV infection is critical in the management of the disease so that anti-viral therapy can be started early. Here we describe the development of real-time PCR assays using TaqMan probes and molecular beacons and compare the performance of both assays with a well-established, validated, gel-based PCR method for the quantification of EBV and CMV in patients’ samples. The TaqMan and molecular beacon assays were linear between 10 to 107 viral genomes/reaction. Both assays generated calibration curves with strong correlation and low intra-assay and interassay variation. Results of EBV and CMV viral load determination inpatient samples obtained by the gel-based and real-time PCR were very similar. The real-time PCR assays showed increases in viral load before clinical measures of viral disease and decreases in viral load during anti-viral therapy in two of six pediatric patients. The data indicate that these TaqMan and molecular beacon approaches are accurate, rapid, and reliable assays for the diagnosis and monitoring of EBV and CMV infections in patients.

http://jmd.amjpathol.org/cgi/content/abstract/6/2/90

The risk of developing second primary cancers is increased in patients with breast cancer. The lung is one of the major target organs, and therefore a differential diagnosis between primary and metastatic cancers is required for the treatment of lung tumors in patients with a history of breast cancer. However, biopsy specimens frequently result in small, fragmented tissues containing only a few, degenerated cancer cells. We attempted to find a useful marker for differential diagnosis, using the online SAGE database. We selected three molecules, small breast epithelial mucin (SBEM), prostate epithelium-specific Ets transcription factor (PDEF), and mammaglobin (MGB1), as potential markers for breast cancer. SBEM and PDEF proved of no use for practical differential diagnosis because they are expressed in the normal bronchus. In contrast, expression of MGB1 was detected in all 22 primary breast cancers, but not in 22 normal lung tissues. Furthermore, all 12 metastatic breast cancers examined demonstrated positive MGB1 transcripts, whereas one of 48 primary lung adenocarcinomas expressed MGB1. This suggests that MGB1 can serve as a differential molecular marker. In practice, prospective examination, using the nine cases with a history of breast cancer, confirmed the usefulness of MGB1 in differential diagnosis.


http://jmd.amjpathol.org/cgi/content/abstract/5/4/237

The clinical management of non-small cell lung cancer (NSCLC) would benefit greatly by a test that was able to detect small amounts of NSCLC in the peripheral blood. In this report, we used a novel strategy to enrich tumor cells from the peripheral blood of 24 stage I to IV NSCLC patients and determined expression levels for six cancer-associated genes (lunx, muc1, KS1/4, CEA, CK19, and PSE). Using thresholds established at three standard deviations above the mean observed in 15 normal controls, we observed that lunx (10 of 24, 42%), muc1 (5 of 24, 21%), and CK19 (5 of 24, 21%) were overexpressed in 14 of 24 (58%) peripheral blood samples obtained from NSCLC patients. Patients who overexpressed either KS1/4 (n = 2) or PSE (n = 1) also overexpressed either lunx or muc1. Of patients with presumed curable and resectable stage I to II disease (n = 7), at least one marker was overexpressed in three (43%) patients. In advanced stage III to IV patients (n = 17), at least one marker was overexpressed in 11 patients (65%). These results provide evidence that circulating tumor cells can be detected in NSCLC patients by a high throughput molecular technique. Further studies are needed to determine the clinical relevance of gene overexpression.


http://jmd.amjpathol.org/cgi/content/abstract/5/4/222

Adenomatous polyposis coli (APC) is a tumor suppressor gene important in colorectal tumorigenesis. A genetic variant of APC, I1307K, results from a T-to-A transversion at nucleotide 3920 which converts the wild-type sequence to a homopolymer tract (A8). The I1307K alteration
is not itself oncogenic, but creates a hypermutable region (A8) that is prone to frame-shift mutations. The APC I1307K variant occurs in approximately 6% of the Ashkenazi Jewish population and is reported to approximately double an individual's risk for colorectal cancer. Here we describe a single nucleotide primer extension assay for the detection of the APC I1307K mutation. Following PCR amplification, nucleotide 3920 of the APC gene is directly sequenced using single nucleotide primer extension technology. The assay is in a multiplex format allowing simultaneous forward and reverse sequencing of the I1307K variant, which provides an internal, independent confirmation of each testing result. The assay was validated against 60 samples previously characterized by an allele-specific oligonucleotide (ASO) hybridization assay, with 100% concordance of results. Compared to the ASO assay, this single nucleotide primer extension assay requires significantly less technical time to perform, and has a greatly increased throughput capacity. The single nucleotide extension assay provides a highly sensitive and specific assay to identify individuals with the APC I1307K gene variant who may benefit from increased colorectal screening.


http://jmd.amjpathol.org/cgi/content/abstract/6/2/137

A multi-site study to assess the accuracy and performance of the biplex Invader assay for genotyping five polymorphisms implicated in venous thrombosis was carried out in seven laboratories. Genotyping results obtained using the Invader biplex assay were compared to those obtained from a reference method, either allele-specific polymerase chain reaction (AS-PCR), restriction fragment length polymorphism (PCR-RFLP) or PCR-mass spectrometry. Results were compared for five loci associated with venous thrombosis: Factor V Leiden, Factor II (prothrombin) G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, and plasminogen activator inhibitor (PAI-1) 4G/5G. Of a total of 1448 genotypes tested in this study, there were 22 samples that gave different results between the Invader biplex assay and the PCR-based methods. On further testing, 21 were determined to be correctly genotyped by the Invader Assay and only a single discrepancy was resolved in favor of the PCR-based assays. The compiled results demonstrate that the Invader biplex assay provides results more than 99.9% concordant with standard PCR-based techniques and is a rapid and highly accurate alternative to target amplification-based methods.


http://jmd.amjpathol.org/cgi/content/abstract/4/1/37

In typical cases of infectious mononucleosis (IM), lymphoid tissue is rarely submitted for pathological examination. When lymphoid tissues from IM cases are examined, the histological appearance of IM may be difficult to distinguish from malignant lymphoma. The purpose of this study was to address the utility of clinical molecular assays for T and B cell clonality in distinguishing IM from lymphoid malignancy. DNA was recovered from paraffin-embedded archival lymphoid tissues of 18 cases of IM and 13 control cases representing other reactive lymphoid hyperplasias. T cell receptor \( \gamma \) (TCR-\( \gamma \)) and immunoglobulin heavy chain (IgH) gene rearrangements were assayed using our standard clinical polymerase chain reaction procedures targeting each of the four functional variable (V) families and the three joining (J) families of the TCR-\( \gamma \) gene, and framework III of the IgH gene, respectively. In 17 of 18 cases of IM, no monoclonal T or B cell populations were detectable. One case, the only spleen specimen in the study, had an oligoclonal pattern of TCR-\( \gamma \) rearrangements. The control
cases representing other reactive hyperplasias also lacked monoclonality. The assays used were sensitive to clonal populations as small as 5% of cells. In this case series, no monoclonal lymphoid populations were identified in any case of IM. This finding suggests that molecular studies are useful in distinguishing IM from lymphoid neoplasms.


http://jmd.amjpathol.org/cgi/content/abstract/4/4/223

Follicular lymphoma is characterized by the presence of the t(14;18)(q32;q21) chromosomal translocation which juxtaposes the bcl-2 gene at 18q21 with the immunoglobulin heavy chain locus at 14q32. Quantification of t(14;18) carrying cells in FL patients can be achieved by real-time PCR, a highly sensitive technique for evaluating treatment efficacy and minimal residual disease. Despite the many advantages of real-time technology for this purpose, one disadvantage is that current real-time t(14;18) PCR assays amplify a control gene as a normalizer in a separate reaction. Since each PCR reaction has its own kinetics, separate PCR assays for target and control sequences can potentially result in inaccurate quantification of t(14;18)-positive cells. In addition, the real-time t(14;18) PCR assays do not determine the size of the amplified fusion sequence, which is helpful for excluding contamination and is commonly used to demonstrate clonal identity between pre- and post-treatment specimens from a patient. To address these limitations, we designed a multiplex real-time PCR protocol that allows amplification of control and target genes in the same reaction and precise size determination of bcl-2/JH fusion sequences by capillary electrophoresis. This multiplex PCR assay is equally sensitive to previous assays, allows more accurate quantification of bcl-2/JH fusion sequences, and is more convenient.


http://jmd.amjpathol.org/cgi/content/abstract/7/2/289

Characterization of CFTR mutations in the U.S. Hispanic population is vital to early diagnosis, genetic counseling, patient-specific treatment, and the understanding of cystic fibrosis (CF) pathogenesis. The mutation spectrum in Hispanics, however, remains poorly defined. A group of 257 self-identified Hispanics with clinical manifestations consistent with CF were studied by temporal temperature gradient electrophoresis and/or DNA sequencing. A total of 183 mutations were identified, including 14 different amino acid-changing novel variants. A significant proportion (78/85) of the different mutations identified would not have been detected by the ACMG/ACOG-recommended 25-mutation screening panel. Over one third of the mutations (27/85) occurred with a relative frequency >1%, which illustrates that the identified mutations are not all rare. This is supported by a comparison with other large CFTR studies. These results underscore the disparity in mutation identification between Caucasians and Hispanics and show utility for comprehensive diagnostic CFTR mutation analysis in this population.

PCR amplification of part of the X-Y homologous amelogenin gene with a single primer pair has been used as a sex identification test because it generates different length products from the X and Y chromosomes. Using a commercially available kit that contains amelogenin primers, we report a single phenotypically normal Caucasian male out of 327 males tested to date that failed to show an X chromosome-specific PCR product. Using alternative amelogenin primers external to but encompassing the initial amplicon, an X chromosome-specific product was seen. Sequence analysis of this X-specific PCR product revealed a C to G mutation at the most 3' base of the initial reverse amelogenin PCR primer. An alternative reverse PCR primer with this most 3' base deleted showed X- and Y-specific products from the case study male. Rare mutations that result in a failure to amplify sex chromosome-specific products can result in incorrect gender identification.


Mastocytosis is characterized by focal heterotypic clusters of mast cells and lymphocytes in the bone marrow and by a somatically acquired activating Kit mutation, D816V. The relationship of the occurrence of this mutation to the heterotypic clusters of mast cells and lymphocytes in bone marrow is unknown. We hypothesized that these two unique features of mastocytosis were related. To explore this hypothesis, laser capture microdissected mast cells, B cells, and T cells, from both lesional and non-lesional areas of bone marrow biopsy tissues from patients with mastocytosis, were examined for the D816V mutation in their DNA, using HinfI restriction digestion of nested PCR products amplified from extracts of dissected cells. The D816V mutation was detected in mast cells, B cells, and T cells from lesional but not non-lesional areas of bone marrow tissues. B cells obtained from lesional areas of tissue were also assessed for clonality and were found to at least represent an oligoclonal population. Thus, mast cells and lymphocytes within focal aggregates in the bone marrow of those with mastocytosis are more frequently positive for the codon 816 activating mutation. Further, the B cell population is oligoclonal, suggesting that clonal proliferation is unlikely to be the basis of clustering.


Several approaches for the detection of minimal residual disease (MRD) in childhood acute lymphoblastic leukemia (ALL) have shown the importance of determining the level of MRD precisely. In the present study, we tested a new real-time quantitative polymerase chain reaction (RQ-PCR) strategy with minor groove binder (MGB) technology for immunoglobulin heavy chain gene rearrangements by positioning a MGB probe at the germline JH segments and one of the primers at the downstream introns in combination with an allele-specific oligonucleotide (ASO) primer complementary to the VH-DH or DH-JH junctional region. A MGB probe forms extremely stable duplexes with single-stranded DNA targets, allowing the use of shorter probes for hybridization-based assays. Therefore, it shows positional flexibility. We have designed two novel consensus MGB JH germline probes for analyzing all of the germline rearrangements registered.
in the V BASE database, and demonstrated that the MRD was detectable with the probes in 17 cases of childhood ALL. The actual copy number for the targets and dynamic changes before and after treatment were almost identical between the JH MGB probe and conventional non-MGB probes in each patient. MGB technology will undoubtedly contribute to MRD-PCR studies of childhood ALL.


http://jmd.amjpathol.org/cgi/content/abstract/5/2/113

Protein tyrosine kinases (PTKs) control key functions of normal and malignant cells. Comparison of PTK gene expression among various cell populations may be achieved by amplification of the PTK cDNAs using degenerate primers which recognize two relatively invariable regions within their catalytic domain. This approach produces a mixture of PTK cDNA fragments with identical or very similar lengths which are difficult to separate by standard gel electrophoresis. These mixed products are then analyzed in a random fashion which leads to redundant cloning of some and potential omission of other PTKs. By using parallel denaturing gradient gel electrophoresis (DGGE) we have been able to separate the amplified PTK cDNAs derived from the same T-lymphocyte population and compare their expression between various types of normal and malignant T lymphocytes. One such PTK is the type I receptor for insulin-like growth factor, which we found to be preferentially expressed by neoplastic T cells on the both mRNA and protein levels. The combination of PCR which uses PTK-specific primers and parallel DGGE of the amplified PTK cDNAs may prove useful in studying mechanisms of cell activation and malignant transformation and in identifying targets for therapies based on selective inhibition of oncogenic PTKs.

Mol. Endocrinol. (18)


http://mend.endojournals.org/cgi/content/abstract/18/1/173

Pituitary adenylate cyclase-activating polypeptide (PACAP) regulates the secretion of GnRH into the hypothalamic hypophysial portal system and sensitizes the pituitary for release of hormones that trigger ovulation. Because reproductive behavior is synchronized with GnRH release, the present study was undertaken to determine whether PACAP in the ventromedial nucleus (VMN) plays a role in receptivity. To this end, we used rat and mouse reproductive behavioral models to determine the biological relationship between PACAP and steroid receptor function in females. We provide evidence for the requirement of PACAP in the VMN for progesterone (P)-dependent sexual behavior in estrogen (E)-primed females. We clarify the biological and molecular mechanisms of PACAP activity by showing 1) that inhibition of endogenous PACAP suppresses P receptor (PR)-dependent sexual behavior facilitated by the steroid P or D1-like agonist SKF38393 and 2) that PR, steroid receptor coactivators-1 and -2, and new protein synthesis are
essential for ligand independent PACAP-facilitated behavior. These findings are consistent with convergence of PACAP-mediated cellular signals on PR for genomic activation and subsequent behavioral changes. Further, we show that steroids regulate both endogenous PACAP mRNA in the VMN and immunoreactive PACAP in the medial basal hypothalamus and cerebral spinal fluid for ligand-dependent, steroid receptor-dependent receptivity. The present findings delineate a novel, steroid-dependent mechanism within the female hypothalamus by which the neuropeptide PACAP acts as a feed-forward, paracrine, and/or autocrine factor for synchronization of behavior coordinate with hypothalamic control of ovulation.


http://mend.endojournals.org/cgi/content/abstract/19/3/621

11{beta}-Hydroxysteroid dehydrogenase type 1 (11{beta}-HSD1) plays an important role in the prereceptor regulation of corticosteroids by locally converting cortisol into active cortisone. To investigate the impact of this mechanism on osteoblast development, we have characterized 11{beta}-HSD1 activity and regulation in a differentiating human osteoblast cell line (SV-HFO). Continuous treatment with the synthetic glucocorticoid dexamethasone induces differentiation of SV-HFO cells during 21 d of culture. Using this cell system, we showed an inverse relationship between 11{beta}-HSD1 activity and osteoblast differentiation. 11{beta}-HSD1 mRNA expression and activity were low and constant in differentiating osteoblasts. However, in the absence of differentiation (no dexamethasone), 11{beta}-HSD1 mRNA and activity increased strongly from d 12 of culture onward, with a peak around d 19. Promoter reporter studies provided evidence that specific regions of the 11{beta}-HSD1 gene are involved in this differentiation controlled regulation of the enzyme. Functional implication of these changes in 11{beta}-HSD1 is shown by the induction of osteoblast differentiation in the presence of cortisone. The current study demonstrates the presence of an intrinsic differentiation-driven molecular switch that controls expression and activity of 11{beta}-HSD1 and thereby cortisol production by human osteoblasts. This efficient mechanism by which osteoblasts generate cortisol in an autocrine fashion to ensure proper differentiation will help to understand the complex effects of cortisol on bone metabolism.


http://mend.endojournals.org/cgi/content/abstract/16/9/2101

RUSH-1{alpha}({beta}) transcription factors were cloned by recognition site screening with an 85-bp region (-170/-55) of the rabbit uteroglobin gene. Deletion analysis showed this region was essential to prolactin (PRL) action, but conclusions were limited by the complexity of the large deletion. Cyclic amplification and selection of targets (CASTing) was used to identify the RUSH-binding site (-126/-121). Endometrial nuclear proteins were incubated with a pool of degenerate oligonucleotides and immunoprecipitated with RUSH-1{alpha}({beta}) antibodies. Bound DNA was amplified by PCR. The consensus motif (MCWTDK) was identified after five rounds of CASTing, authenticated by CASTing with RUSH-1{alpha}-specific antibodies and recombinant protein, and refined with EMSA. Dissociation rate constants (Kd = 0.1-1.0 nM; r = 0.99) revealed high-affinity binding. Chromatin immunoprecipitation confirmed in vivo binding of RUSH to the transcriptionally active uteroglobin promoter. CASTing also revealed RUSH-GATA transcription factor interactions. Endometrial GATA-4 expression is progesterone dependent (Northern analysis) and preferentially localized in the epithelium (in situ hybridization). Although physically
affiliated with RUSH, uterine forms of GATA-4 were not required for RUSH-DNA binding. Site-directed mutagenesis and transient transfection assays showed the RUSH motif mediates the ability of PRL to augment progesterone-dependent uteroglobin transcription. RUSH is central to the mechanism whereby PRL augments progesterone-dependent gene transcription.


http://mend.endojournals.org/cgi/content/abstract/17/10/2070

The physiological responses of the rodent uterus to acute estrogen (E) dosing can be divided into early and late events. Examples of early responses include increased RNA transcription, hyperemia, and water imbibition 2 and 6 h following E administration respectively, whereas later responses include cycles of DNA synthesis and mitosis of epithelial cells beginning 10 and 16 h after E. The development of estrogen receptor (ER) knockout (ERKO) mice, combined with microarray technology, has allowed us to design a genomic approach to study the acute response of the rodent reproductive tract to E. To determine whether early and late biological responses are correlated with altered regulation of a single set of genes or distinct sets of genes characteristic of early and late responses, uterine RNA was obtained from ovariectomized mice that were treated with vehicle or with estradiol for 2 h (early) or 24 h (late). Samples were also prepared from identically treated mice that lacked either ER{alpha} (αERKO) or ER{beta} (βERKO) to address the relative contributions of the ERs in the uterine responses. Microarray analysis of the relative expression of 8700 mouse cDNAs indicated distinct clusters of genes that were regulated both positively and negatively by E in the early or late phases as well as clusters of genes regulated at both times. Both early and late responses by the βERKO samples were indistinguishable from those of WT samples, whereas the αERKO showed little change in gene expression in response to E, indicating the predominant role for ER{alpha} in the genomic response. Further studies indicated that the genomic responses in samples from intermediate time points (6 h, 12 h) fall within the early or late clusters, rather than showing unique clusters regulated in the intermediary period. The use of this genomic approach has illustrated how physiological responses are reflected in genomic patterns. Furthermore, the identification of functional gene families that are regulated by E in the uterus combined with the utilization of genetically altered experimental animal models can help to uncover and define novel mechanisms of E action.


http://mend.endojournals.org/cgi/content/abstract/18/11/2672

Chimeric RET/PTC (rearranged in transformation/papillary thyroid carcinoma) oncoproteins are constitutively active tyrosine kinases found in thyroid papillary carcinoma and nonneoplastic Hashimoto’s thyroiditis. Although several proteins have been identified to be substrates of RET/PTC kinases, the pathogenic roles played by RET/PTC in malignant and benign thyroid diseases and the molecular mechanisms that are involved are not fully understood. We found that RET/PTC expression phosphorylates the Y701 residue of STAT1, a type II interferon (IFN)-responsive protein. RET/PTC-mediated signal transducer and activator of transcription 1 (STAT1) phosphorylation requires RET/PTC kinase activity to be intact but other tyrosine kinases, such as Janus kinases or c-Src, are not involved. RET/PTC-induced STAT1 transcriptional activation was not inhibited by suppressor of cytokine signaling-1 or -3, or protein inhibitors of activated STAT3
([protein inhibitor of activated STAT (PIAS3)], but PIAS1 strongly repressed the RET/PTC-induced transcriptional activity of STAT1. RET/PTC-induced STAT1 activation caused IFN regulatory factor-1 expression. We found that STAT1 and IFN regulatory factor-1 cooperated to significantly increase transcription from type IV IFN- (gamma) responsive promoters of class II transactivator genes. Significantly, cells stably expressing RET/PTC expressed class II transactivator and showed enhanced de novo membrane expression of major histocompatibility complex (MHC) class II proteins. Furthermore, RET/PTC1-bearing papillary thyroid carcinoma cells strongly expressed MHC class II (human leukocyte-associated antigen-DR(alpha)) genes, whereas the surrounding normal tissues did not. Thus, RET/PTC is able to phosphorylate and activate STAT1. This may lead to enhanced MHC class II expression, which may explain why the tissues surrounding RET/PTC-positive cancers are infiltrated with lymphocytes. Such immune response-promoting activity of RET/PTC may also relate to the development of Hashimoto's thyroiditis.


http://mend.endojournals.org/cgi/content/abstract/18/7/1708

Histone acetylation status influences transcriptional activity, and the mechanism of negative gene regulation by thyroid hormone remains unclear, although its impairment by a mutant thyroid hormone receptor (TR) is critical for resistance to thyroid hormone (RTH). We found a novel RTH mutant, F455S, that exhibited impaired repression of the TRH gene and had a strong dominant-negative effect on the gene. F455S strongly interacted with nuclear receptor corepressor (NCoR) and was hard to dissociate from it. To analyze the dynamics of histone acetylation status in vivo, we established cell lines stably expressing the TRH promoter and wild-type or F455S TR. Treatment with a histone deacetylase (HDAC) inhibitor completely abolished the repression of the gene by T3. The histones H3 and H4 at the TRH promoter were acetylated, and addition of T3 caused recruitment of HDACs 2 and 3 within 15 min, resulting in a transient deacetylation of the histone tails. TR and NCoR were located on the promoter, and T3 caused NCoR dissociation and steroid receptor coactivator-1 recruitment. In the presence of F455S, the histones were hyperacetylated, and HDAC recruitment and histone deacetylation were significantly impaired. This is the first report demonstrating the direct involvement of aberrant dynamics of chromatin modification in RTH.


http://mend.endojournals.org/cgi/content/abstract/18/12/3050

Polycystic ovary syndrome (PCOS) represents the most common cause of anovulatory infertility and affects 5-10% of women of reproductive age. The etiology of PCOS is still unknown. The current study is the first to describe consistent differences in gene expression profiles in human ovaries comparing PCOS patients vs. healthy normoovulatory individuals. The microarray analysis of PCOS vs. normal ovaries identifies dysregulated expression of genes encoding components of several biological pathways or systems such as Wnt signaling, extracellular matrix components, and immunological factors. Resulting data may provide novel clues for ovarian dysfunction in PCOS. Intriguingly, the gene expression profiles of ovaries from (long-term) androgen-treated female-to-male transsexuals (TSX) show considerable overlap with PCOS. This observation provides supportive evidence that androgens play a key role in the pathogenesis of PCOS. Presented data may contribute to a better understanding of dysregulated pathways in
PCOS, which might ultimately reveal novel leads for therapeutic intervention.


http://mend.endojournals.org/cgi/content/abstract/16/4/799

Cloning and sequencing of the murine chromosomal region XB harboring the murine vasopressin V2 receptor (mV2R) gene and comparison with the orthologous human Xq28 region harboring the human vasopressin V2 receptor (hV2R) revealed conservation of the genomic organization and a high degree of sequence identity in the V2R coding regions. Despite an identity of 87% of the amino acid sequences, both receptors show marked functional differences upon stable expression in Chinese hamster ovary cells: the mV2R displayed a 5-fold higher affinity for [3H]AVP than the human ortholog; similar differences were found for the AVP-mediated activation of adenylyl cyclase. Saturation binding experiments with transiently transfected intact COS.M6 cells showed that the mV2R was 3- to 5-fold less abundantly expressed at the cell surface than the hV2R. Laser scanning microscopy of fusion proteins consisting of the V2Rs and green fluorescent protein (GFP) (mV2R/GFP, hV2R/GFP) demonstrated that the hV2R/GFP was efficiently transported to the plasma membrane, whereas the mV2R/GFP was localized mainly within the endoplasmic reticulum. Chimeric hV2Rs, in which the first and/or second extracellular loop(s) were replaced by the corresponding loop(s) of the mV2R, revealed that the second extracellular loop accounts for the differences in ligand binding, but the first extracellular loop accounts for the reduced cell surface expression. The exchange of lysine 100 by aspartate in the first extracellular loop of hV2R was sufficient to reduce cell surface expression, which was accompanied by intracellular retention as observed in laser scanning microscopy analysis. Conversely, the exchange of aspartate 100 by lysine in the mV2R increased the cell surface expression and resulted in predominant plasma membrane localization. Thus, a single amino acid difference in the first extracellular loop between mV2R and hV2R determines the efficiency of cell surface expression.


http://mend.endojournals.org/cgi/content/abstract/18/7/1687

We present data suggesting that corticosteroid-binding globulin (CBG) may be the causal gene of a previously identified quantitative trait locus (QTL) associated with cortisol levels, fat, and muscle content in a pig intercross. Because Cbg in human and mouse maps in the region orthologous to the pig region containing this QTL, we considered Cbg as an interesting positional candidate gene because CBG plays a major role in cortisol bioavailability. Firstly, we cloned pig Cbg from a bacterial artificial chromosome library and showed by fluorescent in situ hybridization and radiation hybrid mapping that it maps on 7q26 at the peak of the QTL interval. Secondly, we detected in a subset of the pig intercross progeny a highly significant genetic linkage between CBG plasma binding capacity values and the chromosome 7 markers flanking the cortisol-associated QTL. In this population, CBG capacity is correlated positively to fat and negatively to muscle content. Thirdly, CBG capacity was three times higher in Meishan compared with Large White parental breeds and a 7-fold difference was found in Cbg mRNA expression between the two breeds. Overall, the data accumulated in this study point to Cbg gene as a key regulator of cortisol levels and obesity susceptibility.
Protein tyrosine phosphatases (PTPs) play key roles in switching off tyrosine phosphorylation cascades, such as initiated by cytokine receptors. We have used substrate-trapping mutants of a large set of PTPs to identify members of the PTP family that have substrate specificity for the phosphorylated human GH receptor (GHR) intracellular domain. Among 31 PTPs tested, T cell (TC)-PTP, PTP-{beta}, PTP1B, stomach cancer-associated PTP 1 (SAP-1), Pyst-2, Meg-2, and PTP-H1 showed specificity for phosphorylated GHR that had been produced by coexpression with a kinase in bacteria. We then used GH-induced, phosphorylated GH receptor, purified from overexpressing mammalian cells, in a Far Western-based approach to test whether these seven PTPs were also capable of recognizing ligand-induced, physiologically phosphorylated GHR. In this assay, only TC-PTP, PTP1B, PTP-H1, and SAP-1 interacted with the mature form of the phosphorylated GHR. In parallel, we show that these PTPs recognize very different subsets of the seven GHR tyrosines that are potentially phosphorylated. Finally, mRNA tissue distribution of these PTPs by RT-PCR analysis and coexpression of the wild-type PTPs to test their ability to dephosphorylate ligand-activated GHR suggest PTP-H1 and PTP1B as potential candidates involved in GHR signaling.

Extracellular calcium rapidly controls PTH secretion through binding to the G protein-coupled calcium-sensing receptor (CASR) expressed in parathyroid glands. Very little is known about the regulatory proteins involved in desensitization of CASR. G protein receptor kinases (GRK) and {beta}-arrestins are important regulators of agonist-dependent desensitization of G protein-coupled receptors. In the present study, we investigated their role in mediating agonist-dependent desensitization of CASR. In heterologous cell culture models, we found that the transfection of GRK4 inhibits CASR signaling by enhancing receptor phosphorylation and {beta}-arrestin translocation to the CASR. In contrast, we found that overexpression of GRK2 desensitizes CASR by classical mechanisms as well as through phosphorylation-independent mechanisms involving disruption of G{alpha}q signaling. In addition, we observed lower circulating PTH levels and an attenuated increase in serum PTH after hypocalcemic stimulation in {beta}-arrestin2 null mice, suggesting a functional role of {beta}-arrestin2-dependent desensitization pathways in regulating CASR function in vivo. We conclude that GRKs and {beta}-arrestins play key roles in regulating CASR responsiveness in parathyroid glands.
Stat5b, are major mediators of prolactin signaling in both the mammary gland and in the ovary. Deficiencies in Stat5b, or in both Stat5a and Stat5b, result in loss of pregnancy during midgestation and are correlated with an increase in ovarian 20(alpha)-hydroxysteroid dehydrogenase (20(alpha)-HSD) and a decrease in serum progesterone, which normally declines only immediately before parturition. To determine the relative contribution of 20(alpha)-HSD to progesterone metabolism and Stat5 function during pregnancy and parturition, we created a 20(alpha)-HSD-deficient strain of mice by gene disruption. Mice deficient for 20(alpha)-HSD sustain high progesterone levels and display a delay in parturition of several days demonstrating that 20(alpha)-HSD regulates parturition downstream of the prostaglandin F2(alpha) receptor in an essential and nonredundant manner. Moreover, 20(alpha)-HSD deficiency partially corrected the abortion of pregnancies associated with Stat5b deficiency, supporting the concept that prolactin activation of Stat5b is important in suppressing 20(alpha)-HSD gene expression and thereby allowing the maintenance of progesterone levels that are required to sustain pregnancy.


http://mend.endojournals.org/cgi/content/abstract/18/10/2424

Cholesterol 7-(alpha)-hydroxylase (CYP7A1) catalyzes a rate-limiting step in bile acid synthesis in liver, and its gene transcription is under complex regulation by multiple nuclear receptors in response to bile acids, cholesterol derivatives, and hormones. The liver receptor homolog-1 (LRH-1), a member of the fushi tarazu factor 1 subfamily of nuclear receptors, has emerged as an essential regulator for the expression of cyp7a1. In this report, we demonstrate Prox1, a prospero-related homeobox transcription factor, identified through a yeast two-hybrid screening, can directly interact with human LRH-1 (hLRH-1) and suppresses hLRH-1-mediated transcriptional activation of human cyp7a1 gene. Biochemical analysis demonstrates that Prox1 interacts with both the ligand binding domain (LBD) and the DNA binding domain (DBD) of hLRH-1. An LRKLL motif in Prox1 is important for the interaction with the LBD but not the DBD of hLRH-1. An LRKLL motif in Prox1 is important for the interaction with the LBD but not the DBD of hLRH-1. Gel shift assays reveal that Prox1 impairs the binding of hLRH-1 to the promoter of human cyp7a1 gene.


http://mend.endojournals.org/cgi/content/abstract/18/6/1533

Prostaglandin (PG) E2 E-series prostanoid-2 (EP2) receptor is elevated in numerous carcinomas including the endometrium and has been implicated in mediating the effects of PGE2 on vascular function. In this study, we investigated the intracellular signaling pathways that are activated by the EP2 receptor and their role in regulation of the expression of vascular endothelial growth factor in endometrial adenocarcinoma (Ishikawa) cells. Ishikawa cells were stably transfected with EP2 receptor cDNA in the sense or antisense directions. Treatment of Ishikawa cells with PGE2 rapidly induced transactivation of the epidermal growth factor receptor (EGFR) and activation of ERK1/2 via the EP2 receptor. Preincubation of cells with chemical inhibitors of protein kinase A, c-Src, and EGFR kinase abolished the EP2-induced activation of EGFR and ERK1/2. PGE2
signaling via the EP2 receptor also promoted the mRNA expression and secretion of vascular endothelial growth factor protein in Ishikawa cells. This effect was inhibited by preincubation with chemical inhibitors of EGFR kinase, ERK1/2 signaling, and small inhibitory RNA molecules targeted against the EGFR. Therefore, we have demonstrated that elevated EP2 receptor expression may facilitate the PGE2-induced release of proangiogenic factors in reproductive tumor cells via intracellular cAMP-mediated transactivation of the EGFR and ERK1/2 pathways.


Inhibin is composed of an {alpha}- and a {beta}-subunit. Transgenic studies assigned a tumor-suppressive role to the inhibin {alpha}-subunit, and in human prostate cancer inhibin {alpha}-subunit gene expression was down-regulated. This study examined the inhibin {alpha}-subunit gene promoter and gene locus to determine whether promoter hypermethylation or LOH occurred in DNA from prostate cancer. The 5'-untranslated region of the human inhibin {alpha}-subunit gene was sequenced and shown to be highly homologous to the bovine, rat, and mouse inhibin {alpha}-subunit promoter sequences. A 135-bp region of the human promoter sequence that continued a cluster of CpG sites was analyzed for hypermethylation. Significant (P < 0.001) hypermethylation of the inhibin {alpha}-subunit gene promoter occurred in DNA from Gleason pattern 3, 4, and 5 carcinomas compared with nonmalignant tissue samples. A subset of the carcinomas with a cribriform pattern were unmethylated. LOH at 2q32-36, the chromosomal region harboring the inhibin {alpha}-subunit gene, was observed in 42% of prostate carcinomas. These data provide the first demonstration that promoter hypermethylation and LOH are associated with the inhibin {alpha}-subunit gene and gene locus in prostate cancer.


The insulin receptor substrate-3 (IRS-3) is a member of a family of intermediate adapter proteins that function as major intracellular targets for phosphorylation by the activated insulin and IGF-I receptors. Among the four IRS proteins identified so far, IRS-3 exhibits a rather peculiar expression pattern during both the embryonic development and adult life, suggesting a different mechanism of regulation of its expression. In this study, we cloned the 5' flanking region of the mIRS-3 gene and analyzed its promoter activity. The mIRS-3 promoter is inhibited by wild-type p53, and this effect is completely abolished by cotransfection of a dominant negative p53. Tumor-derived p53 mutants show variable, but lower suppressing capability than wt p53. In addition, treatment with doxorubicin inhibits endogenous expression of mIRS-3 mRNA in C2C12 and 3T3-L1 cells. The DNA region spanning from nucleotides -287 and -178 in the mIRS-3 promoter is responsible for a 32.2% reduction of the mouse double minute 2 (MDM2) promoter activity, suggesting its involvement in the p53-mediated inhibitory effect. In conclusion, our study demonstrates that the mIRS-3 promoter is regulated by p53 at the transcriptional level. The inhibition of mIRS-3 promoter by wild-type p53, and its de-repression by tumor-derived p53 mutants, appears to be similar to that previously reported for the IGF-I receptor promoter, suggesting a common role of these two genes in p53-mediated cell growth and differentiation.
We identified 25 protein tyrosine phosphatases (PTPs) expressed in rat ovarian granulosa cells. Of these PTPs, the expression levels of at least PTP20, PTP-MEG1, PTP(epsilon)M, and PTP(epsilon)C significantly changed during the estrous cycle. We examined the cellular functions of PTP20 in granulosa cells by expressing the wild type, a catalytically inactive CS mutant in which Cys229 of PTP20 was changed to Ser, or a substrate-trapping DA mutant in which Asp197 was mutated to Ala, using an adenovirus vector. Overexpression of the wild type, but not of the CS mutant, induced retraction of the cell body with the extension of long, dendritic-like processes after stimulation with FSH, a critical factor for the survival and differentiation of these cells. In addition, cell adhesion to the substratum decreased in an FSH-dependent manner. Inhibiting Rho GTPase activity with C3 botulinum toxin caused similar morphological changes. The FSH-enhanced phosphotyrosine (p-Tyr) level of p190 RhoGAP was selectively reduced by the overexpressed wild type, but not by mutated PTP20. Although p190 RhoGAP is tyrosine phosphorylated by c-Src via the tyrosine kinase Pyk2, wild-type PTP20 had little effect on p-Tyr418 of c-Src and no effect on p-Tyr402 of Pyk2, which are required for full c-Src activity and for interacting between Pyk2 and c-Src, respectively. The CS and DA mutants as well as the wild type reduced the formation of p190 RhoGAP-p120 RasGAP complexes. Confocal microscopy analysis revealed that PTP20 intracellularly colocalizes with p190 RhoGAP. These results demonstrate that PTP20 regulates the functions of granulosa cells in an FSH-dependent manner by dephosphorylating p190 RhoGAP and subsequently inducing reorganization of the actin cytoskeleton. Moreover, our data suggest that PTPs play significant roles in controlling the dynamics of ovarian functions.

Central nervous system stem cells give rise to neurons and glia when exposed to specific trophic factors. In our studies with rat fetal brain-derived stem cells (RSCs), we showed that they could be induced to express the developmentally regulated transcription factors and cell markers characteristic of cells derived from another germ layer, e.g. pituitary cells. Therefore, rat fetal brain-derived stem cells do not seem to be restricted to a defined developmental fate. They may retain pluripotentiality and can be redirected to develop into other cell types not found in the brain provided the correct set of stimuli is present. This multipotent developmental behavior also suggests that instructive signals are operative.

Association between pre-eclampsia (PEE1) and the dimethylarginine dimethylaminohydrolase (DDAH) 1 and 2 genes, which play a role in the regulation of nitric oxide synthesis and release, was studied. In a case-control study design single nucleotide polymorphisms (SNPs) were determined at eight sites in the DDAH1 gene and at one site (Pro231Pro) in the DDAH2 gene from 132 women with pre-eclampsia and 112 healthy controls. Three SNPs in the DDAH1 gene were associated with pre-eclampsia, showing complete linkage disequilibrium with each other, but none of the associations in the allele or genotype data reached statistical significance in either of the genes after the correction for multiple testing. Haplotype frequencies were estimated using a population based on a maximum likelihood method (EM algorithm). Four common DDAH1 haplotypes were present and a significant association of haplotypes H2 and H3 with pre-eclampsia (P=0.03) was found. The risk of pre-eclampsia was greatest in individuals (odds ratio: 3.93; 95% confidence interval: 1.54-9.99) who had two copies of the high-risk haplotypes (H2 or H3). The observed haplotypic association provides the first evidence of the importance of DDAH1 polymorphisms in pre-eclampsia susceptibility.


The transcription factor OCT-4 is regarded as a critical factor in controlling mammalian early embryonic development because of its role in toti-/pluripotency. In human preimplantation embryos, OCT-4 studies are limited to RNA analysis of abnormally developing embryos. This study thoroughly investigated the expression pattern of OCT-4 throughout the human preimplantation development. Expression was examined by single-cell RT-PCR or indirect immunocytochemistry in 36 single oocytes of various maturity and 112 normally developing preimplantation embryos at the level of single blastomeres, morulas, blastocysts, or inner cell mass (ICM) and trophoderm (TE) samples. Oocytes and cleavage stage embryos revealed a variable OCT-4 expression pattern, concomitant with a pure cytoplasmic localization of the protein. During compaction, the variability in expression faded away indicating embryonic OCT-4 expression and the protein appeared in the nucleus implying biological activity. In blastocysts, OCT-4 transcripts and proteins were present in the ICM and the TE. At protein level, blastocysts displayed different spatial expression patterns within a cell for the splice variants of OCT-4, which may endow them with different functional properties. As OCT-4 transcripts were also found in various differentiated cells, the presence of OCT-4 transcripts or proteins may not be sufficient for identifying undifferentiated cell lines in humans. Further, we suggest to examine the localization of OCT-4 proteins within a cell rather than to look for the presence and/or amount of transcripts.


The mechanisms underlying the switch from uterine quiescence to contractile activity in labour are not clearly understood. Increasing evidence suggests that pathways of myometrial calcium homeostasis, including store-operated calcium entry (SOCE), may play an important role. The molecular basis of the membrane-associated calcium channels contributing to SOCE in pregnant human myometrium is not known, but they are likely to be hetero- or homo-oligomeric assemblies of transient receptor potential channel (TrpC) proteins, encoded by the mammalian homologues.
of Drosophila Trp genes. This study has therefore determined Trp gene expression and also TrpC protein expression and localization in term pregnant human myometrial tissue and primary cultured human myometrial smooth muscle (HMSM) cells. RT-PCR amplified fragments of Trp1, Trp3, Trp4, Trp6 and Trp7. PCR products were 100% homologous to published human sequences. Western blot analysis detected TrpC1, TrpC3, TrpC4 and TrpC6 proteins, which were of expected size. Immunolocalization revealed TrpC1, TrpC3, TrpC4 and TrpC6 protein expression in myometrial tissue and HMSM cells. TrpC protein immunostaining in HMSM cells was distributed in a distinct reticular fashion. TrpC proteins may be candidate proteins forming SOCE channels in term pregnant human myometrium.


http://molehr.oupjournals.org/cgi/content/abstract/9/7/429

Charcot-Marie-Tooth (CMT) disease is the common' name for a range of hereditary peripheral neuropathies. CMT1 is the most common form and is transmitted in an autosomal dominant manner. CMT1A maps to chromosome 17p11.2 and is caused, in the majority of cases, by a 1.5 Mb DNA duplication, that includes the peripheral myelin protein 22 (PMP) gene. This paper reports on preimplantation genetic diagnosis (PGD) for CMT1A in five couples. The CMT1A duplication was detected by fluorescent PCR analysis using polymorphic (CA)n markers localized within the duplication. Single-cell PCR on blastomeres allowed genetic analysis of embryos obtained after ICSI. Only healthy unaffected embryos were transferred to the uterus. PCR experiments with single EBV-transformed lymphoblasts or with research blastomeres allowed the evaluation of amplification efficiencies, as well as contamination and allele drop-out (ADO) rates for each PCR protocol. Three simplex PCR protocols (using one primer pair) and two duplex PCR protocols (using two primer pairs) were developed for CMT1A. Additionally, a protocol using all three primer pairs in triplex was also established. Thirteen clinical ICSI-PGD cycles were performed for five couples (12 simplex PCR cycles and one duplex PCR cycle), resulting in seven embryo transfers. Three singleton pregnancies ensued in two couples and three healthy babies were delivered. This report describes different fluorescent PCR-based tests which allow efficient and accurate single-cell level detection of the CMT1A duplication. On the basis of the presence of the healthy allele of the affected parent-to-be (and/or absence of the affected one), healthy embryos can be selected for transfer. The assays are suitable for PGD for other couples who present with the same CMT1A duplication [depending on their informativity for the (CA)n markers available] as described here.


http://molehr.oupjournals.org/cgi/content/abstract/9/12/793

In mineralocorticoid target tissues, 11{beta}-hydroxysteroid dehydrogenase type 2 (11{beta}-HSD2) confers mineralocorticoid receptor selectivity by metabolizing hormonally active cortisol to inactive cortisone, allowing aldosterone access to the receptor. This enzyme is also expressed in high abundance in fetal tissues, particularly in placental trophoblast, where a role has been proposed in regulating fetal growth and development by protecting the fetus from maternal hypercortisolaemia and modulating local glucocorticoid receptor (GR), rather than mineralocorticoid receptor-mediated responses. As such the placenta has not been considered a mineralocorticoid target tissue. We have used conventional RT-PCR and real-time quantitative RT-PCR to demonstrate that primary cultures of term human cytotrophoblast express the mineralocorticoid-responsive genes Na/K-ATPase (α1 and β1 subunits), epithelial
sodium channel (ENaC, \{alpha\} and \{gamma\} subunits) and the serum and glucocorticoid-inducible kinase (SGK). SGK expression was found to be rapidly and strongly induced by corticosteroids (24- and 38-fold by 10^{-7} \text{ mol/l aldosterone} and 10^{-7} \text{ mol/l dexamethasone respectively after 1 h}). Dexamethasone-, but not aldosterone-stimulated SGK induction was inhibited by GR antagonist (RU38486), confirming the presence of a functional mineralocorticoid receptor and suggesting that placental trophoblast expresses a functional mineralocorticoid receptor, which is in part responsible for the corticosteroid regulation of SGK expression. Placental 11(beta)-HSD2 may protect the MR in a fashion analogous to classical mineralocorticoid tissues to modulate trophoblast sodium transport.


http://molehr.oupjournals.org/cgi/content/abstract/10/6/445

Preimplantation HLA matching has recently emerged as a tool for couples desiring to conceive a potential donor progeny for transplantation in a sibling with a life-threatening disorder. In this paper we describe a strategy optimized for preimplantation genetic diagnosis (PGD) of haemoglobinopathies combined with HLA matching. This procedure involves a minisequencing-based genotyping of HLA regions A, B, C and DRB combined with mutation analysis of the gene regions involved by mutation. Analysis of at least eight polymorphic short tandem repeat (STR) markers scattered through the HLA complex has also been included to detect potential contamination and crossing-over occurrences between HLA genes. The above assay can also be used for preimplantation HLA matching as a primary indication. The strategy was clinically applied for HLA matching in 17 cycles (14 for \{beta\}-thalassaemia, one for Wiscott-Aldrich syndrome and two for leukaemia). A reliable HLA genotype was achieved in 255/266 (95.9\%) of the blastomeres. In total, 22 (14.8\%) embryos were obtained that were HLA-matched with the affected siblings, 14 (9.4\%) of which were unaffected and transferred back to the patients. Four clinical pregnancies were obtained, three of which (one twin, two singletons) are ongoing and were confirmed as healthy and HLA-identical with the affected children. Minisequencing-based HLA typing combined with HLA STR haplotyping has been shown to be a reliable strategy for preimplantation HLA matching. The major advantage of this approach is that the validation of a single assay can be done once and then used for the majority of the patients, reducing notably time needed for preclinical set-up of each case.


http://molehr.oupjournals.org/cgi/content/abstract/9/7/399

We have applied a new method of genetic analysis, called minisequencing', to preimplantation genetic diagnosis (PGD) of monogenic disorders from single cells. This method involves computer-assisted mutation analysis, which allows exact base identity determination and computer-assisted visualization of the specific mutation(s), and thus facilitates data interpretation and management. Sequencing of the entire PCR product is unnecessary, yet the same qualitative characteristics of sequence analysis are maintained. The main benefit of the minisequencing strategy is the use of a mutation analysis protocol based on a common procedure, irrespective of the mutations involved. To evaluate the reliability of this method for subsequent application to PGD, we analysed PCR products from 887 blastomeres including 55 PGD cases of different genetic diseases, such as cystic fibrosis, \{beta\}-thalassaemia, sickle cell anaemia, haemophilia A, retinoblastoma, and spinal muscular atrophy. Minisequencing was found to be a useful technique
in PGD analysis, due to its elevated sensitivity, automation, and easy data interpretation. The method was also efficient, providing interpretable results in 96.5% (856/887) of the blastomeres tested. Fifteen clinical pregnancies resulted from these PGD cases; conventional prenatal diagnosis confirmed all the PGD results, and 10 healthy babies have already been born. Its applicability to PGD could be helpful, particularly in cases in which the mutation(s) involved are difficult to assess by restriction analysis or other commonly used methods.


http://molehr.oupjournals.org/cgi/content/abstract/11/3/195

Controlled ovarian hyperstimulation (COH) used in IVF produces lower implantation rates per embryo transferred compared to natural cycles utilized in ovum donation, suggesting a suboptimal endometrial development. Endometrial receptivity has recently been investigated in natural menstrual cycles with the aid of microarray technology. The aim of this study is to investigate the impact of COH using urinary gonadotrophins with a long protocol with GnRH agonists without progesterone supplementation (similar to the natural cycle) on endometrial gene expression profiles during the window of implantation by comparing the profiles at day hCG+7 of COH versus LH+7 of a previous natural cycle in the same women. For this purpose we have used microarray technology by Affymetrix (GeneChip HG_U133A), which allows more than 22,000 genes to be tested simultaneously. Results were validated by semi-quantitative PCR and quantitative PCR experiments. We found that more than 200 genes showed a differential expression of more than 3-fold when COH and normal cycles were compared at hCG+7 versus LH+7. We simultaneously re-analysed the LH+2 versus LH+7 endometrial gene expression profiles in previous natural cycles in the same subject using this specific GeneChip, the results obtained were consistent with our own published results. This is the first time that gene expression profiles of the endometrium during COH are reported. The large degree of gene expression disturbance is surprising and highlights the need for further efforts to optimize COH protocols.


http://molehr.oupjournals.org/cgi/content/abstract/10/12/901

Stromal cell-derived factor-1 (SDF-1) or CXCL12 is the physiologic ligand for the chemokine receptor CXCR4. CXCR4-mediated signalling regulates cell migration and apoptosis in certain haematopoietic and neuronal cells. Using gene profiling, we determined that CXCR4 is the only chemokine receptor for which mRNA expression is regulated during trophoblast differentiation in vitro. Based on the known effects of CXCR4 ligation, we hypothesized that CXCR4 activation may regulate placental trophoblast cell survival (i.e. protection from apoptosis), an important mechanism for the establishment and maintenance of the uteroplacental barrier. Human cytотrophoblasts (CTBs) were cultured in defined media and treated with graded doses of SDF-1 (10-100 ng/ml) or with an anti-CXCR4 neutralizing antibody. Exposure to anti-CXCR4 antibody reduced CTB cell numbers by 25-40%. Treatment with SDF-1 decreased the proportions of apoptotic terminal deoxynucleotidyl transferase-mediated dUTP-FITC nick-end labelling (+) cells (apoptotic index [AI] of 2.79(+/÷)0.61 % [control] versus 1.88(+/÷)0.56 % [SDF-1]; P<0.05) and caspase-activated cells (AI of 7.95(+/÷)2.49 % [control] versus 3.81(+/÷)1.49 % [SDF-1]; P<0.05). We determined that SDF-1 also activated the triple MAP Kinase isoforms ERK1/2 and p38 in trophoblasts. Immunocytochemistry confirmed SDF-1-induced nuclear translocation of phosphorylated ERK1/2. Blocking of ERK1/2 signalling with the specific inhibitor PD98059
reversed SDF-1-mediated inhibition of apoptosis (AI of 1.65{+/-}0.34 [SDF-1] versus 3.50{+/-}0.5 [SDF-1 + PD98059]; P<0.05), suggesting that SDF-1 acts through this pathway as a trophoblast survival factor. These results indicate that SDF-1/CXCR4 signalling stimulates anti-apoptotic pathways in cultured trophoblasts. This chemotactic ligand/receptor system may promote trophoblast survival during pregnancy. Alterations in SDF-1 and/or CXCR4 expression or function may be associated with specific pregnancy disorders.


http://molehr.oupjournals.org/cgi/content/abstract/11/2/93

Hypospadias is one of the most common congenital anomalies. Increased exposure to environmental factors (endocrine-disrupting chemicals and smoking) or maternal endogenous estrogen may cause hypospadias because male sexual differentiation is dependent on normal androgen homeostasis. Moreover, interactions between genetic factors and cigarette smoking and other chemicals have been suggested. It has been demonstrated that the CYP1A1 metabolizes not only environmental chemicals but also estrogens, and glutathione-S-transferases (GSTs) are detoxification enzymes that protect cells from toxicants by conjugation with glutathione. In this study, to investigate the association of CYP1A1 (MspI), GSTM1 and GSTT1 polymorphisms with hypospadias, a case-control study of 31 case mothers who had boys with hypospadias and 64 control mothers was performed in Japan. These polymorphisms were investigated by PCR-based methods using DNA from peripheral lymphocytes. We found that the heterozygous CYP1A1 and heterozygous and homozygous CYP1A1 were less frequent in the case mothers than in the control mothers [adjusted odds ratio (OR)=0.17, 95% confidence interval (CI)=0.04-0.74, OR = 0.28, 95% CI = 0.08-0.97, respectively]. We found no effect of maternal smoking on the hypospadias risks among the gene polymorphisms. The results suggest that mothers with the CYP1A1 MspI variant allele may have a decreased risk for hypospadias.


http://molehr.oupjournals.org/cgi/content/abstract/10/12/895

Labour is associated with increased synthesis of interleukin-8 (IL-8) by the fetal membranes and myometrium, which leads to an inflammatory infiltrate. Stretch has been shown to increase the expression of contraction-associated proteins in animal models of labour and in human myocytes in vitro. In this study, we tested the hypothesis that mechanical stretch of human myometrial cells increases IL-8 messenger ribonucleic acid (mRNA) expression. We isolated myocytes from non-pregnant women undergoing hysterectomy and pregnant women undergoing Caesarean section before and after the onset of labour. Myocytes in culture were subjected to stretch of varying intensity (6-16%) and duration (1 or 6 h) using the Flexercell system. IL-8 mRNA expression was lowest in myocytes from pregnant women not in labour, intermediate in those from non-pregnant women and greatest in those from pregnant women in labour. Stretch increased IL-8 mRNA expression independent of reproductive state. The stretch-induced increase in IL-8 mRNA expression was associated with higher IL-8 levels in the culture supernatant and enhanced promoter activity. These data suggest that stretch contributes to the increase in myometrial IL-8 synthesis associated with the onset of labour in humans.

http://molehr.oupjournals.org/cgi/content/abstract/9/6/345

Human semen coagulum predominantly consists of high molecular mass complexes of the seminal vesicle secreted semenogelin I (SgI) and semenogelin II (SgII). Here we describe a previously unknown variant of the SgI gene that is present at an allele frequency of ~3% in the Swedish population. It gives rise to a protein with a molecular mass of 43 kDa, SgI43, which compared with the 50 kDa variant, SgI50, is lacking a tandem repeat of 60 amino acid residues that was probably deleted by homologous recombination. In spite of the size difference, SgI43 has many properties in common with SgI50, such as a very high iso-electric point and susceptibility to proteolytic degradation by prostate-specific antigen. Heterozygous carriers of the SgI43 allele neither show impaired fertility nor do they significantly differ from individuals homozygous for SgI50 with respect to sperm parameters such as semen volume, sperm count and fraction of motile spermatozoa.


http://molehr.oupjournals.org/cgi/content/abstract/11/2/129

SLC26A8 is an anion transporter that is solely expressed in the testes. It interacts with MgcRacGAP that shows strong structural similarity with the Drosophila protein RotundRacGAP, which is established to have an essential role for male fertility in the fruit fly. To explore whether the SLC26A8 gene has a role in human male infertility, we performed mutational analysis in the coding region of the SLC26A8 gene in 83 male infertility patients and two groups of controls using single-strand conformational polymorphism and direct sequencing methods. We found six novel coding sequence variations, of which five lead to amino acid substitutions. All variants were found with similar frequencies in both patients and controls, thus suggesting that none of them may be causally associated with infertility. We conclude that the SLC26A8 mutations are not a common cause of male infertility.


http://molehr.oupjournals.org/cgi/content/abstract/9/7/411

Preimplantation genetic diagnosis (PGD) of single gene disorders relies on PCR-based tests performed on single cells (polar bodies or blastomeres). Despite the use of increasingly robust protocols, allele drop-out (ADO; the failure to amplify one of the two alleles in a heterozygous cell) remains a significant problem for diagnosis using single cell PCR. In extreme cases ADO can affect >40% of amplifications and has already caused several PGD misdiagnoses. We suggest that an improved understanding of the origins of ADO will allow development of more reliable PCR assays. In this study we carefully varied reaction conditions in >3000 single cell amplifications, allowing factors influencing ADO rates to be identified. ADO was found to be affected by amplicon size, amount of DNA degradation, freezing and thawing, the PCR programme, and the number of cells simultaneously amplified. Factors found to have little or no affect on ADO were local DNA sequence, denaturing temperature (94 or 96(degrees)C) and cell
type. Consideration of the causal factors identified during this study should permit the design of PGD protocols that experience little ADO, thus improving the accuracy of PGD for single gene disorders.


http://molehr.oupjournals.org/cgi/content/abstract/8/7/688

Two healthy sisters with a familial history of mental retardation were referred to our centre for preimplantation genetic diagnosis (PGD). Their two brothers showed severe mental retardation. The molecular basis for their disorder could not be identified, but one of the sisters and the mother presented a highly skewed pattern of X-inactivation reinforcing the likelihood of an X-linked mode of inheritance. Both sisters requested PGD to avoid the abortion of potentially affected male fetuses. PGD for sex by fluorescent in-situ hybridization was carried out for the first sister and resulted in the birth of a female child. The second sister and her partner, whose niece had cystic fibrosis (CF), were tested for CF mutations, and were both found to be (Delta)F508 heterozygous. We developed an efficient single cell PCR protocol for the simultaneous amplification of the CF ((Delta)(Delta)F508) locus as well as the X-linked amelogenin gene and its highly homologous pseudogene on the Y chromosome. Two PGD cycles were carried out to screen against male and (Delta)F508 homozygous deleted embryos. In each case several embryos could be selected for transfer and the second cycle resulted in a twin pregnancy followed by the birth of two healthy female infants.


http://molehr.oupjournals.org/cgi/content/abstract/8/12/1065

Enhanced cyclooxygenase (COX) expression and prostaglandin E2 (PGE2) synthesis are regarded as promoters of neoplastic cell proliferation and angiogenesis. Expression of COX-2 and synthesis of PGE2 are up-regulated in cervical carcinomas. In sexually active women, growth and invasiveness of neoplastic cervical epithelial cells may be also under the direct influence of PGE2 present in seminal plasma. The aims of this study were to investigate the effect of seminal plasma and PGE2 on the expression of COX-2 and expression and signalling of the PGE2 receptor subtypes (EP1-EP4) in HeLa (cervical adenocarcinoma) cells. Treatment of HeLa cells with seminal plasma or PGE2 resulted in up-regulation of COX-2 expression (P < 0.05). In addition, seminal plasma induced the mRNA expression of EP1, EP2 and EP4 receptors, whilst PGE2 treatment of HeLa cells induced the expression of the EP4 receptor (P < 0.05). This was coincident with a rapid accumulation of adenosine 3',5'-cyclic monophosphate (cAMP) in HeLa cells stimulated with seminal plasma or PGE2, which was greater in seminal plasma stimulated cells compared with PGE2 stimulated cells (P < 0.05). Subsequently, we investigated whether the effect of seminal plasma on cAMP signalling in HeLa cells was mediated via the cAMP-linked EP2/EP4 receptors. Stimulation of HeLa cells with seminal plasma or PGE2 resulted in an augmented cAMP accumulation in cells transfected with the EP2 or EP4 receptor cDNA compared with control transfected cells (P < 0.05). These data suggest that, in sexually active women, seminal plasma may play a role in modulating neoplastic cell function and cervical tumorigenesis.
The aetiology of recurrent pregnancy loss (RPL) remains unclear, but it may be related to a possible genetic predisposition together with involvement of environmental factors. We examined the relation between RPL and polymorphisms in two genes, glutathione S-transferases (GST) M1 and T1, which are involved in the metabolism of a wide range of environmental toxins and carcinogens. A case-control study of 115 cases with RPL and 160 controls was conducted. All cases and controls were women resident in Sapporo, Japan and the surrounding area. They were genotyped for polymorphisms of GSTM1 and GSTT1 using PCR-based methods. We found that 65.2% of the cases with RPL and 45.6% of the controls had the GSTM1 null genotype [odds ratio (OR) = 2.23, 95% confidence interval (CI) = 1.36-3.66]. On the other hand, 47.0% of the cases and 49.4% of the controls had the GSTT1 null genotype (OR = 0.95; 95% CI = 0.58-1.55). The results suggest that women with GSTM1 null polymorphism may therefore have an increased risk of RPL.

The CYP17 gene encodes the enzyme cytochrome P450c17\(\alpha\), which mediates both 17\(\alpha\)-hydroxylase and 17,20-lyase activity in the steroid biosynthesis pathway. A T\(\rightarrow\)C polymorphism in the 5' promoter region of CYP17 has been described. To examine the association between recurrent pregnancy loss (RPL) and a polymorphism in CYP17, a case-control study of 117 cases with RPL and 164 controls was conducted. This polymorphism was investigated by PCR/restriction fragment length polymorphism using DNA from peripheral lymphocytes. The T\(\rightarrow\)C transition in the variant allele (A2) creates a new recognition site for the restriction enzyme MspA1, which permits designation of the wildtype allele (A1) and A2. Women with the A2 allele of CYP17 had an increased risk of RPL [A1/A1 genotype (reference); A1/A2 genotype: odds ratio (OR), 1.68; 95% confidence interval (CI), 0.94-3.01; A2/A2 genotype: OR, 2.37; 95% CI, 1.16-4.83; P trend, 0.016]. Additionally, there was a similar tendency for the increased risk of primary RPL [A1/A1 genotype (reference); A1/A2 genotype: OR, 2.14; 95% CI, 1.14-4.01; A2/A2 genotype: OR, 2.50; 95% CI, 1.16-5.41; P trend, 0.015]. These results suggest that possession of the A2 variant of CYP17 may predispose to an increased risk of RPL with a gene dosage effect.

The Y chromosome-specific gene SRY is one of the key genes involved in human sex determination. The SRY gene encodes a testis-specific transcription factor that plays a key role in sexual differentiation and development in males and is located on the distal region of the short arm of the Y chromosome. Mutations in SRY gene result in XY sex reversal and pure gonadal
dysgenesis. SRY expression initiates a network of gene activity that transforms the undifferentiated gonad, genital ridge into testis. Mutations in the SRY gene have been considered to account for only 10-15% of 46,XY gonadal dysgenesis cases, whereas the majority of the remaining cases may have mutation(s) in the SRY regulatory elements or other genes involved in the sex differentiation pathway. Patients both with gonadal dysgenesis and Y-chromosome presence are at high risk of developing gonadoblastoma. Using PCR, single strand conformational polymorphism (SSCP) and automated DNA sequencing, we analysed the mutations in the SRY gene in three 46,XY sex reversal patients. Two patients demonstrated nucleotide substitution (A→G) within the open reading frame just outside and upstream of the conserved DNA-binding motif called the high-mobility group (HMG) box, replacing glutamine at codon 57 with arginine. Altered SSCP patterns were also observed in these patients. Histological examination of gonads in patient 1 revealed the formation of gonadoblastoma. Patient 3 demonstrated A→T substitution which replaces serine at codon 143 with cysteine, just outside but downstream of the HMG box. Results suggest the involvement of SRY gene in sex reversal which further supports the relationship between SRY alterations, gonadal dysgenesis and/or primary infertility.

http://molehr.oupjournals.org/cgi/content/abstract/9/11/701

The expression of Gas6, the protein product of the growth arrest-specific gene 6 (gas6), a member of the vitamin K-dependent protein family, and its receptor tyrosine kinases Axl and Sky and their mRNAs in uterine leiomyoma and normal uterine myometrium tissues were investigated by competitive RT-PCR-Southern blot analysis using recombinant RNA and immunohistochemical analysis respectively. There was no significant difference between the histoscores and levels of Sky mRNA in uterine leiomyoma and normal uterine myometrium, although the levels of Gas6 and Axl mRNAs in uterine leiomyoma were significantly higher than in normal uterine myometrium in each case. It is suggested that Gas6 and Axl signal transduction is aberrantly stimulated in uterine leiomyoma, possibly related to its growth.

http://molehr.oupjournals.org/cgi/content/abstract/8/6/552

We demonstrated the expression of Gas6, the protein product of the growth arrest-specific gene 6 (gas6) and a member of the vitamin K-dependent protein family, and its receptor tyrosine kinases, Axl and Sky, in human uterine and ovarian endometriotic endometria using RT-PCR-Southern blot analysis and immunohistochemistry. Gas6, Axl and Sky mRNA were detected in all samples analysed. There was no significant difference between the levels of Sky mRNA in normal uterine and endometriotic endometria; however, the levels of Gas6 and Axl mRNA in endometriotic endometria were significantly higher than in normal endometria. These mRNA levels showed no significant alteration during the menstrual cycle. In the immunohistochemical study, Gas6, Axl and Sky were found in endometrial glandular cells and stromal cells in all samples analysed. This study demonstrates the coexpression of receptor tyrosine kinases and their ligand, Gas6, in normal uterine and ovarian endometriotic endometria, and the overexpression of Axl and Gas6 in endometriotic endometria. It is suggested that Gas6 and Axl signal transduction is aberrantly stimulated in endometriotic endometria, and is plausibly related
Steroid hormone receptor co-factors are abundantly expressed in the uterus in order to modify steroid hormone receptor action, either leading to activation or repression of transcription in the endometrium. However, the role of co-factors in remodelling of the human endometrium has not been established. We therefore endeavoured to evaluate the presence of the co-activator SRC (steroid receptor co-activator)-1 and the co-repressors N-CoR (nuclear receptor co-repressor) and steroid co-repressor SMRT (silencing mediator of retinod and thyroid) receptors in the human endometrium during the different phases of the menstrual cycle. By using a real-time RT-PCR assay, we showed that SRC-1, N-CoR and SMRT mRNA are expressed in human endometrium during all phases of the menstrual cycle, as well as in inactive endometrium. Moreover, endometrial expression of SRC-1 and N-CoR mRNA increased during menstruation when compared with the other phases of the menstrual cycle (P < 0.001). Immunohistochemistry demonstrated that SRC-1 and N-CoR stain positive in the glandular epithelium and stroma in menstrual phase endometrium. The staining was weak in proliferative and secretory endometrium and absent in inactive endometrium. Our results suggest that differential expression of endometrial steroid receptor co-factors probably play a role in the regulation of human endometrium remodelling.

Intrauterine fetal growth restriction is a multifactorial disorder, and its aetiology includes both environmental and genetic components. We aimed to investigate whether maternal genetic polymorphisms of metabolic enzymes affects fetal growth and pregnancy duration. Genomic DNA was obtained from 134 women who experienced singleton deliveries beyond 24 weeks of gestation. Maternal age, birth weight, gestational age at birth and frequencies of fetal growth restriction, prematurity and pregnancy-induced hypertension were compared among genotypic subgroups of cytochrome P450 (CYP) and glutathione S-transferase (GST) genes. The polymorphisms of CYP1A1 (MspI), CYP17 (MspAl) and GSTP1 (BsmAl) genotypes, and the presence or absence of GSTM1 and GSTT1 genes were analysed by PCR-based methods. The frequency of fetal growth restriction (<10th percentile/-1.5 SD; 22.7%/11.4%) in 44 women who were homozygous for the A1 allele (A1A1) of CYP17 was significantly higher than that (7.8%/2.2%) in 90 women who carried the A2 allele (A1A2/A2A2) of CYP17 (P < 0.05), with an odds ratio =3.41 (95% confidence interval = 1.18-9.84). The gestational age at birth (mean +/- SD, 37.5 +/- 3.1 weeks) in 67 women with GSTM1 null genotype was significantly lower than that (38.5 +/- 2.4 weeks) in 67 women who carried GSTM1 (P < 0.05). The polymorphism of CYP17 that encodes the cytochrome P450c17(α) enzyme might be associated with the pathophysiology underlying fetal growth restriction.

http://mp.bmjjournals.com/cgi/content/abstract/56/1/43

Background: The application of lymphoscintigraphy followed by sentinel lymph node (SN) biopsy to patients with primary melanoma has revolutionised the ability to identify accurately, yet conservatively, those patients who harbour occult nodal metastases. The molecular detection of SN micrometastases facilitates the cost effective analysis of the entire SN using multiple markers. Currently, a lack of marker specificity is the main barrier preventing the molecular evaluation of SN tissue from becoming clinically applicable. Aims: To develop a reproducible multimarker reverse transcription-polymerase chain reaction (RT-PCR) assay, with the emphasis on achieving high specificity for the accurate detection of melanoma metastases in nodal tissue. Methods: Three pigment cell specific (PCS) markers—tyrosinase, Pmel-17, and MART-1—and one cancer testis antigen (CTA)—MAGE-3—were selected for use in a multimarker RT-PCR assay. The conditions for this assay were optimised. Results: High specificity was achievable for each marker by optimising the PCR cycle number such that unwanted transcripts (that is, illegitimate transcripts and/or specific transcripts from other low abundance nodal cell types) remained undetectable in appropriate controls (normal visceral nodes). Tyrosinase was 100% specific at 40 PCR cycles, MAGE-3 and MART-1 at 35 PCR cycles, and Pmel-17 at 30 PCR cycles. Tyrosinase proved to be the most sensitive marker, detecting 10 melanoma cells in 0.1 g of nodal tissue. Conclusions: Excellent reproducibility of the entire nodal processing and RT-PCR protocol for the detection of very low numbers of melanoma cells in nodal tissue was shown, although there is a risk of false positives using the PCS markers alone, because of an approximate 4-8.5% incidence rate of nodal nevi in melanoma draining SNs (these nevi being absent in all other normal nodes). MAGE-3 was shown to be the only marker that is not expressed by melanocytes. However, because not all melanomas express MAGE-3, it is recommended that more emphasis should be placed on the development of a panel of CTA markers to ensure a zero false positive rate and to provide optimum detection.


http://mp.bmjjournals.com/cgi/content/abstract/55/1/55

CpG islands are GC rich sequences that are found in the promoters of many genes in higher eukaryotes. They contain a high frequency of CG dinucleotides, which are substrates for DNA methylases. Methylation leads to transcriptional silencing of promoters. Owing to their high GC content CpG islands exhibit strong base-base interactions, which lead to superstructures and consequently to regions with higher melting temperatures. Therefore, Taq polymerases (especially sequenases) fall off their templates, causing premature termination of the polymerase chain reaction (PCR) or sequencing reactions. The results from such reactions are thus insufficient for further analysis. Therefore, we have evaluated the use of 7-deaza-2`-deoxyguanosine for PCR amplification of the human p16INK4A promoter and sequencing of HUMARA exon 1 PCR products. Our results show that the addition of 7-deaza-2`-deoxyguanosine significantly improves results, particularly when small amounts of poor quality DNA are available as starting material.
Background/Aims: Rearrangement of immunoglobulin gene segments, leading to B cells with functional receptors, is thought to be largely restricted to developing immature B cells in bone marrow. However, accumulating evidence suggests that mature B cells occasionally modify their antigen specificity by VH segment replacements during the germinal centre reaction to enhance antigen affinity, or to overcome self reactive antigen receptors. Although malignant B cells maintain the features of their normal counterparts in most instances, to date, such replacements have not been described for human B cell lymphomas. Methods: Rearranged immunoglobulin heavy chain genes from two extranodal marginal zone B cell lymphomas were amplified, cloned, and sequenced. Sequences with identical CDR3 regions were selected and aligned to each other and public databases. Results: VH replacements were seen in two extranodal marginal zone B cell lymphomas. In line with the hypothesis that in mature B cells these replacements are associated with active somatic hypermutation, in addition to VH replacement, different mutation patterns were seen in the revised VH portions. In the remaining common 3’-VH regions, these mutations could be used to establish a phylogenetic relation between the sequences, rendering the possibility of artefactual chimaeric polymerase chain reaction products very unlikely. Conclusions: These results support the view that VH replacements are a further mechanism for reshaping antigen affinity and specificity, and indicate that these receptor modifications are not restricted to normal and reactive germinal centre B cells, but may also occur in close association with the development of malignant B cell lymphomas.

Mol. Pharmacol. (18)


The signaling pathways that lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) use to activate Akt in ovarian cancer cells are investigated here. We show for the first time, with the use of both pharmacological and genetic inhibitors, that the kinase activity and S473 phosphorylation of Akt induced by LPA and S1P requires both mitogen-activated protein (MAP) kinase kinase (MEK) and p38 MAP kinase, and MEK is likely to be upstream of p38, in HEY ovarian cancer cells. The requirement for both MEK and p38 is cell type- and stimulus-specific. Among 12 cell lines that we tested, 11 respond to LPA and S1P and all of the responsive cell lines require p38 but only nine of them require MEK. Among different stimuli tested, platelet-derived growth factor stimulates S473 phosphorylation of Akt in a MEK- and p38-dependent manner. However, epidermal growth factor, thrombin, and endothelin-1-stimulated Akt S473 phosphorylation require p38 but not MEK. Insulin, on the other hand, stimulates Akt S473 phosphorylation independent of both MEK and p38 in HEY cells. T308 phosphorylation stimulated by LPA/S1P requires MEK but not p38 activation. MEK and p38 activation were sufficient for Akt S473 but not T308 phosphorylation in HEY cells. In contrast to S1P and PDGF, LPA requires Rho
for Akt S473 phosphorylation, and Rho is upstream of phosphatidylinositol 3-kinase (PI3-K). LPA/S1P-induced Akt activation may be involved in cell survival, because LPA and S1P treatment in HEY ovarian cancer cells results in a decrease in paclitaxel-induced caspase-3 activity in a PI3-K/MEK/p38-dependent manner.


http://molpharm.aspetjournals.org/cgi/content/abstract/66/6/1662

The B cell, a major component of humoral immunity, is a sensitive target for the immunotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), possibly by rendering cells less responsive to antigenic or mitogenic stimulation. Potential mechanisms of TCDD action on B cells were examined in murine B cell lymphoma cells (CH12.LX) treated with 3 nM TCDD or dimethyl sulfoxide vehicle using sequence-verified cDNA microarrays. One transcript that was significantly induced by TCDD was suppressor of cytokine signaling 2 (Socs2). Changes in Socs2 mRNA levels paralleled that of Cyp1a1 with a maximal 3-fold induction observed at 4 h, as determined by quantitative real-time polymerase chain reaction. Socs2 induction seems B cell-specific, because no induction was observed in TCDD-responsive mouse hepatoma cells or human breast cancer cells. TCDD-mediated induction of Socs2 mRNA was dose-dependent and exhibited the characteristic structure-activity relationships observed for the aryl hydrocarbon receptor (AhR) ligands 3,3',4,4',5-pentachlorobiphenyl (PCB-126), indolo[3,2-b]-carbazole, and (beta)-naphthoflavone. Experiments with cycloheximide and AhR-deficient B cells indicated that Socs2 mRNA induction is a primary effect that is AhR-dependent. Western blot analysis confirmed that Socs2 and Cyp1a1 protein levels were also induced in CH12.LX cells. Promoter analysis revealed the presence of four dioxin-response elements within 1000 base pairs upstream of the Socs2 transcriptional start site, and a reporter gene regulated by the Socs2 promoter was inducible by TCDD. Promoter activity was also dependent on a functional AhR signaling pathway. These results indicate that Socs2 is a primary TCDD-inducible gene that may represent a novel mechanism by which TCDD elicits its immunosuppressive effects.


http://molpharm.aspetjournals.org/cgi/content/abstract/63/3/742

We have investigated the sensitivity of the cisplatin-resistant enterohpatic tumor cell lines LS174T/R (human colon adenocarcinoma), WIF-B9/R (rat hepatoma-human fibroblast hybrid), and Hepa 1-6/R (mouse hepatoma) to free and liposome-encapsulated cytostatic bile acid derivatives Bamet-R2 and bamet-UD2. Expression of resistance associated genes was measured by quantitative reverse transcription-polymerase chain reaction or Western blotting. Drug uptake was determined by atomic absorption spectrophotometry. In resistant cells, overexpression of MRP1 and MRP2 was accompanied by reduced accumulation of cisplatin. The expression of MDR1 and GST-P was only enhanced in LS 174T/R. A higher expression of p53 was seen in LS 174T/R and Hepa 1-6/R cell lines but not in WIF-B9/R cells. In wild-type counterparts, uptake and cytostatic ability of Bamets were markedly higher (UD2 > R2) than that of cisplatin. Both effects were further enhanced by liposome formulation. Bamets were able to overcome cisplatin resistance in all cell lines. Cisplatin prolonged the survival time of nude mice in whose livers a Hepa 1-6 tumor had been implanted, but failed to exert a beneficial effect when the tumor was Hepa 1-6/R. In both cases, tissue distribution of cisplatin was: kidney [IMG] liver > tumor. Survival was markedly longer in animals receiving Bamet-UD2, even if the implanted tumor was resistant.
The accumulation of Bamet-UD2 in tissues was: liver > tumor > kidney. Liposome formulation further enhanced the beneficial properties of Bamet-UD2. Thus, the amount of drug in the tumor was increased and that in liver and kidney was reduced (tumor > liver > kidney), and life span was prolonged. In conclusion, liposomal Bamet-UD2 may be a useful tool to circumvent resistance to chemotherapy, particularly in tumors of the enterohepatic circuit.


http://molpharm.aspetjournals.org/cgi/content/abstract/63/1/53

One of the pharmacological targets of ethanol is the GABAA receptor (GABAR), whose function and expression are altered after chronic administration of ethanol. The details of the changes differ between experimental models. In the chronic intermittent ethanol (CIE) model for alcohol dependence, rats are exposed to intermittent episodes of intoxicating ethanol and withdrawal, leading to a kindling-like state of behavioral excitability. This is accompanied by presumably causal changes in GABAR expression and physiology. The present study investigates further the effect of CIE on GABAR function and expression. CIE is validated as a model for human alcohol withdrawal syndrome (AWS) by demonstrating increased levels of anxiety; diazepam improved performance in the test. In addition, CIE rats showed remarkably reduced hypnotic response to a benzodiazepine and a steroid anesthetic, reduced sensitivity to a barbiturate, but not propofol. Immunoblotting revealed decrease in [alpha]1 and [delta] expression and increase in [gamma]2 and [alpha]4 subunits in hippocampus of CIE rats, confirmed by an increase in diazepam-insensitive binding for ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo(1,5-[alpha])(1,4)benzodiazepine-3-carboxylate (Ro15-4513). Elevated mRNA levels were shown for the [gamma]2S and [gamma]1 subunits. Recordings in hippocampal slices from CIE rats revealed that the decay time of GABAR-mediated miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal cells was decreased, and potentiation of mIPSCs by positive modulators of GABAR was also reduced compared with control rats. However, mIPSC potentiation by the [alpha]4-preferring benzodiazepine ligands bretazenil and Ro15-4513 was maintained, and increased, respectively. These data suggest that specific alterations in GABAR occur after CIE and may underlie the development of hyperexcitability and ethanol dependence.


http://molpharm.aspetjournals.org/cgi/content/abstract/65/6/1405

The human xenobiotic-metabolizing enzyme cytochrome P450, CYP2A6, catalyzes the bioactivation of a number of carcinogens and drugs and is overexpressed in cases of liver diseases, such as cirrhosis, viral hepatitis, and parasitic infestation, and in certain tumor cells. This suggests that CYP2A6 may be a major liver catalyst in pathological conditions. In the present study, we have addressed molecular mechanisms underlying the regulation of the CYP2A6 gene. We present evidence of several proteins present in human hepatocytes that interact specifically with the 3'-untranslated region (UTR) of CYP2A6 mRNA. Biochemical and immunological evidence show that the RNA-protein complex of highest intensity contains the heterogeneous nuclear ribonucleoprotein (hnRNP) A1 or a closely related protein. Mapping of the hnRNP A1 binding site within CYP2A6 3'-UTR reveals that the smallest portion of RNA supporting significant binding consists of 111 central nucleotides of the 3'-UTR. Our studies also indicate that hnRNP A1 from HepG2 cancer cells exhibits modified binding characteristics to the
CYP2A6 3'-UTR compared with primary hepatocytes. We found that the level of CYP2A6 mRNA remains high in conditions of impaired transcription in primary human hepatocytes, showing that CYP2A6 expression can be affected post-transcriptionally in conditions of cellular stress. Our results indicate that the post-transcriptional regulation involves interaction of the hnRNP A1 protein with CYP2A6 mRNA. The present data suggest that hnRNPA1 is a critical regulator of expression of the human CYP2A6 gene and support the notion that this P450 isoform may be of particular significance in stressed human liver cells.


http://molpharm.aspetjournals.org/cgi/content/abstract/62/2/334

Nicotine-stimulated 86Rb+ efflux and [3H]cytisine binding, both of which seem to measure the nicotinic acetylcholine receptor, composed of [alpha]4 and [beta]2 subunits, were assessed in eight brain regions obtained from 14 inbred mouse strains. The potential role of a single nucleotide polymorphism (SNP) in the nicotinic receptor [alpha]4 subunit gene (Chrna4) on nicotinic receptor binding and function in mice was also evaluated. This SNP leads to an alanine-to-threonine variation at amino acid position 529 of the nascent [alpha]4 subunit polypeptide. Both nicotine-stimulated 86Rb+ efflux and [3H]cytisine binding were found to vary across brain regions and among mouse strains. Variability in nicotine-stimulated 86Rb+ efflux was positively correlated (r > 0.9) within each strain with the number of [3H]cytisine binding sites. However, the number of [3H]cytisine binding sites was not correlated with nicotine-stimulated 86Rb+ efflux across mouse strains. In contrast, the Chrna4 polymorphism was associated with receptor function across mouse strains: 86Rb+ efflux was greater in seven of the eight brain regions studied in those mouse strains that carry the Ala-529 variant of Chrna4. The Chrna4 SNP did not seem to influence the number of [3H]cytisine binding sites across mouse strains. These data indicate that inbred mouse strains exhibit differences in receptor function that cannot be attributed to variation in receptor expression but may be explained, at least in part, by the missense polymorphism in the [alpha]4 subunit.


http://molpharm.aspetjournals.org/cgi/content/abstract/66/2/260

Identification of the specific muscarinic acetylcholine receptor (mACChR) subtypes mediating stimulation of salivary secretion is of considerable clinical interest. Recent pharmacological and molecular genetic studies have yielded somewhat confusing and partially contradictory results regarding the involvement of individual mACChRs in this activity. In the present study, we re-examined the roles of M1 and M3 mACChRs in muscarinic agonist-mediated stimulation of salivary secretion by using M1 and M3 receptor single-knockout (KO) mice and newly generated M1/M3 receptor double-KO mice. When applied at a low dose (1 mg/kg, s.c.), the muscarinic agonist pilocarpine showed significantly reduced secretory activity in both M1 and M3 receptor single-KO mice. However, when applied at higher doses, pilocarpine induced only modestly reduced (5 mg/kg, s.c.) or unchanged (15 mg/kg, s.c.) salivation responses, respectively, in M1 and M3 receptor single-KO mice, indicating that the presence of either M1 or M3 receptors is sufficient to mediate robust salivary output. Quantitative reverse transcriptase-polymerase chain reaction studies with salivary gland tissue showed that the inactivation of the M1 or M3 mACChR genes did not lead to significantly altered mRNA levels of the remaining mACChR subtypes. Strikingly, the sialagogue activity of pilocarpine was abolished in M1/M3 receptor double-KO mice. However, salivary glands from M1/M3 receptor double-KO mice remained responsive to stimulation by the
A \{\beta}\text{-adrenergic receptor agonist, (S)-isoproterenol. Taken together these studies support the concept that a mixture of M1 and M3 receptors mediates cholinergic stimulation of salivary flow.


http://molpharm.aspetjournals.org/cgi/content/abstract/66/2/337

We reported recently that interleukin (IL)-1{\beta} exposure resulted in a prolonged increase in MUC5AC mucin production in normal, well differentiated, human tracheobronchial epithelial (NHTBE) cell cultures, without significantly increasing MUC5AC mRNA (Am J Physiol 286:L320-L330, 2004). The goal of the present study was to elucidate the signaling pathways involved in IL-1{\beta}-induced MUC5AC production. We found that IL-1{\beta} increased cyclooxygenase-2 (COX-2) mRNA expression and prostaglandin (PG) E2 production and that the COX-2 inhibitor celecoxib suppressed IL-1{\beta}-induced MUC5AC production. Addition of exogenous PGE2 to NHTBE cultures also increased MUC5AC production and IL-1{\beta}-induced Muc5ac hypersecretion in tracheas from wild-type but not from COX-2-/- mice. NHTBE cells expressed all four E-prostanoid (EP) receptor subtypes and misoprostol, an EP2 and EP4 agonist, increased MUC5AC production, whereas sulprostone, an EP1 and EP3 agonist, did not. Furthermore, specific protein kinase A (PKA) inhibitors blocked IL-1{\beta} and PGE2-induced MUC5AC production. However, neither inhibition of epidermal growth factor receptor (EGFR) activation with the tyrosine kinase inhibitor 4-(3-chloroanilino)-6,7-dimethoxyquinazoline HCl (AG-1478) or EGFR blocking antibody nor inhibition of extracellular signal-regulated kinase/P-38 mitogen activated protein kinases with specific inhibitors blocked IL-1{\beta} stimulation of MUC5AC mucin production. We also observed that tumor necrosis factor (TNF)-{\alpha}, platelet activating factor (PAF), and lipopolysaccharide (LPS) induced COX-2 and increased MUC5AC production that was blocked by celecoxib, suggesting a common signaling pathway of inflammatory mediator-induced MUC5AC production in NHTBE cells. We conclude that the induction of MUC5AC by IL-1{\beta}, TNF-{\alpha}, PAF, and LPS involves COX-2-generated PGE2, activation of EP2 and/or EP4 receptor(s), and cAMP-PKA-mediated signaling.


http://molpharm.aspetjournals.org/cgi/content/abstract/61/5/1124

The clinical abuse of methamphetamine (METH) is a major concern because it can cause long-lasting neurodegenerative effects in humans. Current concepts of the molecular mechanisms underlying these complications have centered on the formation of reactive oxygen species. Herein, we provide cDNA microarray evidence that METH administration caused the induction of c-Jun and of other members involved in the pathway leading to c-Jun activation [stress-activated protein kinase/Jun N-terminal kinase (JNK3), Crk-associated substrate-Cas and c-Src] after environmental stresses or cytokine stimulation. Reverse transcription-polymerase chain reaction analysis confirmed these increases and also showed that the expression of JNK1 and JNK3 but not JNK2 was also increased in the METH-treated mice. Western blot analysis showed that METH increased the expression of c-Jun phosphorylated at serine-63 and serine-73 residues. Other upstream members of the JNK pathway, including phosphorylated JNKs, mitogen-activated protein kinase kinase 4, mitogen-activated protein kinase kinase 7, Crk II, Cas, and c-Src were also increased at the protein level. These values returned to baseline by 1 week after drug treatment. These results are discussed in terms of their support for a possible role of the activation of the JNK/Jun pathway in the pathophysiological effects of METH.
Endogenous and xenobiotic-enhanced oxidative stress may initiate embryonic death and birth defects via reactive oxygen species (ROS) signaling pathways involving nuclear transcription factor-{kappa}B (NF-{kappa}B). Using embryo culture and a transgenic mouse engineered with a NF-{kappa}B-dependent beta-galactosidase reporter gene, we employed NF-{kappa}B antisense oligonucleotide therapy to determine whether NF-{kappa}B signaling contributes to the embryopathic effects of the ROS-initiating teratogen phenytoin. Phenytoin selectively increased NF-{kappa}B activity in target tissues and caused embryopathies, both of which were blocked by NF-{kappa}B antisense oligonucleotides but not by sense and nonsense oligonucleotide controls. NF-{kappa}B signaling may therefore contribute to the mechanism of ROS-mediated embryopathies.

CYP2J2 is abundant in cardiovascular tissue and active in the metabolism of arachidonic acid to eicosanoids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties. We cloned and sequenced the entire CYP2J2 gene (~40.3 kb), which contains nine exons and eight introns. We then sequenced the CYP2J2 exons and intron-exon boundaries in 72 healthy persons representing African, Asian, and European/white populations as part of the National Institutes of Health/National Institute of Environmental Health Sciences Environmental Genome Single Nucleotide Polymorphism Program. A variety of polymorphisms were found, four of which resulted in coding changes (Arg158Cys, Ile192Asn, Asp342Asn, and Asn404Tyr). A fifth variant (Thr143Ala) was identified by screening a human heart cDNA library. All five variant cDNAs of CYP2J2 were generated by site-directed mutagenesis and expressed in Sf9 insect cells by using a baculovirus system. The recombinant wild-type and variant CYP2J2 proteins immunoreacted with peptide-based antibodies to CYP2J2 and displayed typical cytochrome P450 (P450) CO-difference spectra; however, the Asn404Tyr and Ile192Asn variants also had prominent spectral peaks at 420 nm. The ability of these variants to metabolize arachidonic acid and linoleic acid was compared with that of wild-type CYP2J2. Three variants (Asn404Tyr, Arg158Cys, and Thr143Ala) showed significantly reduced metabolism of both arachidonic acid and linoleic acid. The Ile192Asn variant showed significantly reduced activity toward arachidonic acid only. The Asp342Asn variant showed similar metabolism to wild-type CYP2J2 for both endogenous substrates. Based on these data, we conclude that allelic variants of the human CYP2J2 gene exist and that some of these variants result in a P450 protein that has reduced catalytic function. Insofar as CYP2J2 products have effects in the cardiovascular system, we speculate that these variants may be functionally relevant.
Cytotoxic platinum compounds including cisplatin are standard cancer chemotherapeutics and are also activators of stress-signaling pathways. In this study, we tested the role of the c-Jun N-terminal kinase (JNK) family of mitogen-activated protein kinases and their transcription factor target, c-Jun, in the cytotoxic response of small-cell lung cancer (SCLC) cells to cisplatin and its less effective trans-isomer, transplatin. Both agents stimulated JNK activity; the transplatin response was rapid and transient, whereas JNK activation by cisplatin was delayed and sustained. Despite the differential kinetics of JNK activation, expression of nonphosphorylatable JNK mutants sensitized the SCLC cells to killing by cisplatin or transplatin, suggesting that JNK activation in response to these agents signals a protective response. Consistent with this finding, overexpression of the JNK target, c-Jun, significantly protected SCLC cells from platinum compounds, whereas expression of a c-Jun mutant encoding only the DNA binding domain increased the sensitivity of the SCLC cells to these drugs. These findings support the hypothesis that activation of the JNKs by platinum compounds controls c-Jun-dependent transcriptional events that promote a protective response in SCLC cells. Oligonucleotide array analysis identified genes encoding a variety of signaling proteins whose expression was reciprocally changed by c-Jun and c-Jun-DBD (c-Jun-DNA binding domain). It is noteworthy that genes whose products are involved in DNA repair, glutathione synthesis, or drug accumulation did not exhibit altered expression by c-Jun or c-Jun-DBD. The findings indicate that inhibition of the JNK pathway is a potential means to enhance the sensitivity of SCLC cells to platinum compounds.


Glucocorticoids are potent anti-inflammatory and immunosuppressant agents. However, they also produce serious side effects that limit their usage. It has been proposed that anti-inflammatory properties of glucocorticoids are caused mostly by repression of activator protein 1- and nuclear factor [kappa][beta]-stimulated synthesis of inflammatory mediators, whereas most of their adverse effects are associated with trans-activation of genes involved with metabolic processes. Our laboratories have sought to discover novel glucocorticoid receptor (GR) ligands that have high repression but low trans-activation activities. We describe here cellular properties of 2,5-dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5-(1-methylcyclohexen-3-y1)-1H-[1]benzopyrano[3,4-f]quinoline (A276575) and its four enantiomers. Similar to dexamethasone, A276575 exhibited high affinity for GR and potently repressed interleukin (IL) 1[beta]-stimulated IL-6 production in human skin fibroblasts, prostaglandin (PG) E2 production in A549 human lung epithelial cells, and concanavalin A-induced monocyte proliferation. In contrast to dexamethasone, A276575 caused smaller induction of aromatase activity in human skin fibroblasts and antagonized dexamethasone-induced activation of an mouse mammary tumor virus-glucocorticoid-response element (GRE) reporter gene construct. Among the four enantiomers of A276575, the two ([-])-enantiomers showed 10- to 30-fold higher affinities for GR than their respective (+)-enantiomers. Both ([-])-Syn and ([-])-Anti enantiomers of A276575 were potent inhibitors of IL-1[beta]-stimulated PGE2 production in A549 lung epithelial cells; unexpectedly, however, only the ([-])-Anti enantiomer inhibited regulated on T-cell activation, normal T-cell expressed and secreted (RANTES) production in A549 cells. In summary, A276575 is a novel, nonsteroidal GR ligand that possesses high repression activities against inflammatory mediator production but has lower GRE trans-activation activities than traditional steroids. Differential repression of RANTES and PGE2 production in a cell by the two ([-])-enantiomers of A276575 illustrates the complexity of repression by GR.

http://molpharm.aspetjournals.org/cgi/content/abstract/65/3/730

Mouse CYP2J5 is abundant in kidney and active in the metabolism of arachidonic acid to epoxyeicosatrienoic acids. Western blots of microsomes prepared from mouse kidneys demonstrate that after puberty, CYP2J5 protein is present at higher levels in male mice than in female mice. Northern analysis reveals that CYP2J5 transcripts are more abundant in adult male versus female kidneys, indicating that gender differences in renal CYP2J5 expression are regulated at a pretranslational level. Castration of male mice results in decreased renal CYP2J5 expression, and treatment of castrated male mice or female mice with 5(alpha)-dihydrotestosterone increases expression to levels that approximate those in intact male mice. In contrast, treatment of ovariectomized female mice or castrated male mice with 17(beta)-estradiol causes a further reduction in CYP2J5 expression. Growth hormone-deficient (lt/lit) mice respond similarly to castration and 5(alpha)-dihydrotestosterone treatment, indicating that the androgen effects are not mediated by alterations in the growth hormone secretory pattern. Mice that lack a functional androgen receptor (Tfm hemizygous) have reduced levels of renal CYP2J5 and do not respond to 5(alpha)-dihydrotestosterone treatment. Similarly, wild-type male mice treated with flutamide, an androgen antagonist, exhibit reduced renal CYP2J5 levels. Female estrogen receptor-(alpha) knockout (alpha ERKO) mice, which are known to have elevated circulating testosterone levels, have significantly increased renal CYP2J5 expression compared with wild-type female mice, and these differences are abrogated by ovariectomy or treatment with flutamide. Based on these data, we conclude that the renal expression of CYP2J5 is up-regulated by androgen and down-regulated by estrogen.


http://molpharm.aspetjournals.org/cgi/content/abstract/66/5/1083

Corticotropin-releasing factor (CRF) plays a central role in the regulation of the hypotalamic-pituitary-adrenal axis, mediating endocrine and behavioral responses to various stressors. Two high-affinity receptors for CRF have been described. Although many of the intracellular signaling pathways activated by CRF have been studied extensively, our knowledge of transcriptional responses downstream of the CRF receptor 1 (CRFR1) is still limited. To elucidate gene networks regulated by CRF and CRFR1, we applied microarray technology to explore transcriptional response to CRF stimulation. Therefore, mouse pituitary-derived AtT-20 cells were exposed continuously to CRF either in the presence or absence of the specific CRFR1 antagonist R121919. Transcriptional responses to different treatments were studied in a time course ranging from 0.5 to 24 h. Microarray data were analyzed using classic microarray data analysis tools such as correspondence factor analysis, cluster analysis, and fold-change filtering. Furthermore, spectral map analysis was applied, a recently introduced unsupervised multivariate analysis method. A broad and transient transcriptional response to CRF was identified that could be blocked by the antagonist. This way, several known CRF-induced target genes and novel CRF responsive genes were identified. These include transcription factors such as cAMP-responsive element modulator (7x increased), secreted peptides such as cholecystokinin (1.5x), and proteins involved in modulating intracellular signaling, such as regulator of G-protein signaling 2 (11x). Up-regulation of many of these genes can be explained as negative feedback, attenuating CRF-activated pathways. In addition, spectral map analysis proved to be a promising new tool for microarray data analysis.

http://molpharm.aspetjournals.org/cgi/content/abstract/66/3/572

Tumor necrosis factor (TNF) receptor 1-associated death domain protein (TRADD) is an adaptor protein known to be involved in the TNF signaling pathway as well as signaling of other members of the TNF receptor superfamily, including DR3, DR6, p75NTR, and the Epstein-Barr virus latent membrane protein 1. Current knowledge of the function of the adaptor protein has been derived from studies examining its over-expression in either wild-type or mutated forms. In this study, we analyzed the consequences of antisense oligonucleotide (ASO)-mediated depletion of endogenous TRADD on TNF induction of inflammation-related gene products, such as intercellular adhesion molecule-1, and associated kinase signaling pathways in human umbilical vein endothelial cells. A broader perspective of TRADD's role in TNF signaling was indicated by microarray gene expression analysis, where 20 of 24 genes that showed a 5-fold or greater increase in TNF-induced mRNA expression levels displayed a reduction in TNF-induced expression as a consequence of ASO-mediated knockdown of TRADD. Reduced activation of the nuclear factor-{kappa}B and c-Jun NH2-terminal kinase pathways, as measured by I{kappa}B-{alpha} protein levels and the extent of c-Jun phosphorylation, was also observed. These results indicate usage of antisense inhibitors of TRADD expression for modulating diseases associated with TRADD-dependent signal transduction pathways.


http://molpharm.aspetjournals.org/cgi/content/abstract/62/2/423

Interaction of two members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family with the 37untranslated region (UTR) of the murine inducible nitric-oxide synthase (iNOS) mRNA is demonstrated in this study. An iNOS RNA-protein complex is formed using protein extracts from untreated and septic shock treated mouse liver. UV cross-linking reveals that the complex consists of at least two proteins, with apparent molecular masses of 60 and 70 kDa, respectively. The 60-kDa protein binding site lies within a 112-nt pyrimidine-rich sequence, approximately 160 nt from the coding sequence, and the RNA-protein complex can be precipitated by a monoclonal antibody directed against hnRNP I [also named polypyrimidine tract binding protein (PTB)]. The 70-kDa protein binds a 43-nt sequence near the 37end of the 37UTR and is immunoprecipitated by a monoclonal antibody against hnRNP L. A computer-simulated conformation of the 37UTR suggests that both binding sites reside in regions easily accessible for a protein. Supershifts of the native RNA-protein complex could only be achieved with anti-hnRNP L, suggesting that within this multiprotein RNA complex, only hnRNP L is exposed to the antibodies, whereas the hnRNP I/PTB is mainly responsible for its interaction with the mRNA. Up-regulation of iNOS by septic shock reduces the RNA-protein complex formation, thus showing that hnRNP I/PTB and hnRNP L binding to the iNOS mRNA is modulated by inflammation. This suggests a novel function for the two previously described proteins as regulators of the iNOS gene.

Struckmann, N., S. Schwering, et al. (2003). "Role of Muscarinic Receptor Subtypes in the Constriction of
In the airways, increases in cholinergic nerve activity and cholinergic hypersensitivity are associated with chronic obstructive pulmonary disease and asthma. However, the contribution of individual muscarinic acetylcholine receptor subtypes to the constriction of smaller intrapulmonary airways that are primarily responsible for airway resistance has not been analyzed. To address this issue, we used videomicroscopy and digital imaging of precision-cut lung slices derived from wild-type mice and mice deficient in either the M1 (mAChR1-/- mice), M2 (mAChR2-/- mice), or M3 receptor subtype (mAChR3-/- mice) or lacking both the M2 and M3 receptor subtypes (mAChR2/3-/- double-knockout mice). In peripheral airways from wild-type mice (mAChR+/+ mice), muscarine induced a triphasic concentration-dependent response, characterized by an initial constriction, a transient relaxation, and a sustained constriction. The bronchoconstriction was diminished by up to 60% in mAChR3-/- lungs and was completely abolished in mAChR2/3-/- lungs. The sustained bronchoconstriction was reduced in mAChR2-/- bronchi, and, interestingly, the transient relaxation was absent; the bronchoconstriction in response to 10-8 M muscarine was increased by 158% in mAChR1-/- mice. Quantitative reverse transcriptase-polymerase chain reaction analysis revealed that the disruption of specific mAChR genes had no significant effect on the expression levels of the remaining mAChR subtypes. These results demonstrate that cholinergic constriction of murine peripheral airways is mediated by the concerted action of the M2 and M3 receptor subtypes and suggest the existence of pulmonary M1 receptor activation, which counteracts cholinergic bronchoconstriction. Given the important role of muscarinic cholinergic mechanisms in pulmonary disease, these findings should be of considerable therapeutic relevance.

**Molecular and Cellular Neuroscience (7)**


Rat CNTF was expressed in Escherichia coli using a T7 RNA polymerase vector system. rCNTF was obtained as an insoluble aggregate at levels approaching 70% of total E. coli protein. After extraction, renaturation, and purification by anion-exchange chromatography and gel filtration the yield of biologically active rCNTF was nearly 0.5 mg/ml of E. coli culture broth. The effects of rCNTF and bFGF on cultured chick ciliary neurons were compared. bFGF was primarily neuritiogenic and did not support ciliary neuron survival beyond 2 days in culture. rCNTF induced neurogenesis more slowly and supported ciliary neuron survival in culture for a longer period. bFGF strongly potentiated the effect of rCNTF. Without bFGF, the ED50 for rCNTF was 102 +/- 25 pg/ml. With bFGF, the potency of rCNTF increased to an ED50 of 42 +/- 6 pg/ml. Chick nodose neurons also responded to rCNTF and their response was potentiated by bFGF. Thus, bFGF modulates the response of ciliary and nodose neurons to CNTF in vitro and may have a similar effect in vivo.

http://www.sciencedirect.com/science/article/B6WNB-48FK4SW-7/2/39ba92863fa0ad23b241cef2152eaf6b

Reactive astrocytes respond to central nervous system (CNS) injury and disease by elaborating a glial scar that is inhibitory to axonal regeneration. To identify genes that may be involved in the astrocytic response to injury, we used differential display polymerase chain reaction and an in vivo model of the CNS glial scar. Expression of the trabecular meshwork inducible glucocorticoid response (TIGR) gene was increased in gliotic tissue compared with the uninjured cerebral cortex. Increased TIGR expression by reactive astrocytes was confirmed by in situ hybridization, quantitative reverse transcriptase-polymerase chain reaction, immunoblot analysis, and immunohistochemistry. Although mutations of the TIGR gene have been implicated in glaucoma, a function for TIGR has not been reported. Since TIGR is secreted, we assessed a possible role in inhibition of neuronal regeneration with an in vitro bioassay and found that this protein is a potent inhibitor of neurite outgrowth. Thus, TIGR is a newly identified component of the CNS glial scar that is likely to contribute to neuronal regenerative failure characteristic of the mammalian CNS.


http://www.sciencedirect.com/science/article/B6WNB-48PVDG8-N/2/6234126ecff62831690858a7dc289d96

Rat peripheral nerve Schwann cells have been shown to express the [alpha]-chemokine receptor CXCR4 as well as the corresponding ligand stromal cell-derived factor-1 (SDF-1). We have investigated gene regulatory mechanisms acting on the expression of CXCR4 in cultured rat Schwann cells and found that receptor expression at transcript- and protein levels is directly dependent on intracellular cyclic AMP. Such increased levels of CXCR4 expression were found to be efficiently reversed by the action of tumor necrosis factor-[alpha] (TNF[alpha]). We also provide evidence that the POU box transcription factor Oct-6/SCIP is involved in the control of CXCR4 transcription. Finally, we could demonstrate that CXCR4 activation by SDF-1[alpha] increases the number of dying Schwann cells, indicating that this receptor/ligand interaction is modulating cell survival. Our data, therefore, suggest that in the Schwann cell lineage signal transduction cascades controlled by the activation of TNF- and CXCR4 receptors are functionally coupled.


http://www.sciencedirect.com/science/article/B6WNB-496NKDJ-6/2/cb8e3b6d6cf762646fc5db398bf100479

SEMA3F is a secreted semaphorin that affects axon and cell guidance in the developing nervous system, and is also thought to have anti-tumor activity. Two spliced forms of SEMA3F have been identified that differ by the insertion of 31 amino acids in the sema domain. Here, we investigated the bioactivity of these isoforms and show, using coculture and binding assays, that they share
common axonal chemorepulsive properties and binding to neuropilin receptors. SEMA3F isoforms were also found to regulate endothelial cell morphology by remodeling lamellipodial protrusions. Although Sema3F expression globally decreased during mouse development, we noted an enrichment of the longest isoform at postnatal stages in some territories such as the brainstem and spinal cord. These results indicate that although functionally redundant in cell culture assays, Sema3F spliced forms are characterized in vivo by a temporal and regional specific regulation during maturation of the nervous system.

http://www.sciencedirect.com/science/article/B6WNB-4DYM8XC-9/2/bbad97d573982c1a79e06b591989b2ca

Recent studies have identified two alternatively spliced forms of the GABAA receptor [gamma]2 subunit that differ by the presence ([gamma]2L) or absence ([gamma]2S) of an eight-amino acid segment. This insert in the [gamma]2L isoform exists in the proposed cytoplasmic loop region, between M3 and M4, and contains a consensus sequence for phosphorylation by protein kinase C. To examine the regional distribution of this novel receptor subunit in the brain, [gamma]2L subunit mRNA was detected using both in situ hybridization histochemistry and and PCR amplification methods. Hybridization histochemistry with a [gamma]2L, subunit-specific oligonucleotide probe revealed that the [gamma]2L, subunit mRNA is widely distributed throughout the mouse brain. The highest levels of expression are found in the cerebral cortex, hippocampus, olfactory lobe, and cerebellum. The presence of the [gamma]2L, subunit in these regions was confirmed using PCR. Additionally, PCR experiments detected yes subunit mRNA in the cerebral cortex and hippocampus but not in the cerebellum. To examine the functional properties of the [gamma]2 subunit isoforms, [gamma]2S and [gamma]2L, subunit mRNAs were coexpressed with [alpha]1[beta]1 subunit mRNAs in Xenopus oocytes. These experiments indicate that the [gamma]2L and [gamma]2S subunit variants exhibit similar pharmacological properties, including the ability of both isoforms to confer diazepam sensitivity to the receptor complex. In addition, potentiation of GABA responses by pentobarbital in oocytes expressing either subunit isoform is similar. These data indicate that the presence or absence of the additional eight amino acids in the [gamma]2 subunit isoforms does not appear to alter the response of the GABAA receptor complex to either benzodiazepines and barbiturates at the level of protein phosphorylation present in the oocyte.

http://www.sciencedirect.com/science/article/B6WNB-48FK4SW-1/2/e9eed170a04bd49b549f60c56a56a301

The p38 mitogen-activated protein kinase (p38MAPK) is activated via phosphorylation in neurones and glial cells by a variety of stimuli including oxidative stress, excitotoxicity, and inflammatory cytokines. Activated p38MAPK can in turn induce phosphorylation of cytoskeletal proteins and activation of cytokines and nitric oxide, thus contributing to neurodegeneration. We investigated the expression and distribution of p38MAPK in the spinal cord of transgenic mice expressing a superoxide dismutase 1 mutation (SOD1G93A), a model of familial amyotrophic lateral sclerosis (ALS). Accumulation of p38MAPK was found by immunoblotting in the spinal cord of G93A mice during the progression of disease, but no changes were detected in its mRNA
levels. Immunostaining for phosphorylated p38MAPK in lumbar spinal cord sections of SOD1G93A mice at the presymptomatic and early stages of disease showed an increased labeling in motor neurones that colocalized with phosphorylated neurofilaments in vacuolized perikarya and neurites, as detected by confocal microscopy. As the disease progressed, activated p38MAPK also accumulated in hypertrophic astrocytes and reactive microglia, as demonstrated by colocalization with GFAP and CD11b immunostaining, respectively. These data suggest that activation of p38MAPK in motor neurons and then in reactive glial cells may contribute, respectively, to the development and progression of motor neuron pathology in SOD1G93A mice.


http://www.sciencedirect.com/science/article/B6WNB-4DXB9YN-1/2/3462dadefb819d5de0299a9b73992ba2

The human 7-transmembrane receptor GPR7 has sequence similarity to opioid and somatostatin receptors, and can be activated by the recently discovered neuropeptides NPB and NPW. This receptor is highly expressed in the nervous system, with suggested roles in neuroendocrine events and pain signaling. In this study, we investigated whether the GPR7 receptor is expressed in the peripheral nervous system under normal and pathological conditions. A low level of GPR7 receptor was observed in myelin-forming Schwann cells in both normal human and rat nerve, and in primary rat Schwann cell cultures. Peripheral nerve samples taken from patients exhibiting inflammatory/immune-mediated neuropathies showed a dramatic increase of GPR7 receptor expression restricted to myelin-forming Schwann cells. Complementary animal models of immune-inflammatory and ligation-induced nerve injury and neuropathic pain similarly exhibited an increased myelin-associated expression of GPR7 receptor. These results suggest a relationship between the pathogenesis of inflammatory/immune-mediated neuropathies, GPR7 receptor expression, and pain transmission.

Molecular and Cellular Probes(38)


http://www.sciencedirect.com/science/article/B6WNC-4CHRGDY-1/2/0fc1a263987e61e7c08ada24452448da

Mutations at embB gene codons 306 and 497 and iniA gene codon 501 occur frequently in ethambutol (EMB)-resistant Mycobacterium tuberculosis strains worldwide. The identification of these mutations in resistant strains has been achieved by labor-intensive DNA sequencing or by tedious amplification protocols followed by restriction endonuclease digestion. In this report, we describe PCR-restriction fragment length polymorphism (RFLP)-based methods for determining substitutions at embB codons 306 and 497 and iniA codon 501 directly in BACTEC cultures of M. tuberculosis isolates. The wild-type and mutant alleles are revealed by easily interpretable and different RFLP patterns. The methods optimized initially on reference strains were tested directly
on BACTEC cultures of 25 randomly selected clinical M. tuberculosis isolates, seven of which were determined to contain EMB-resistant strains by phenotypic drug susceptibility testing. The PCR-RFLP methods identified mutations in four of seven EMB-resistant strains with three isolates containing mutated embB codon 306 and one isolate containing mutated embB codon 497. The results of PCR-RFLP were confirmed by DNA sequencing. The worldwide prevalence figures for mutations at embB codons 306 and 497 and inIA codon 501 suggest that nearly half of EMB-resistant M. tuberculosis strains could be identified within one working day even in developing countries equipped with simple PCR technology instead of weeks required for phenotypic drug susceptibility testing. Further, since EMB resistance is also associated with multiple-drug resistance from some geographical locations, detection of EMB resistance may also lead to rapid identification of multidrug-resistant strains of M. tuberculosis.


http://www.sciencedirect.com/science/article/B6WNC-4DYN471-30/2/5e63dbdc394136ce873a50ed261eeed

As the polymerase chain reaction (PCR) can be used for the generation of vector-free probes, the optimum conditions for incorporation of digoxigenin-11-dUTP into hepatitis B virus (HBV) probes have been investigated. High yields of double-stranded or single-stranded probes can be obtained by utilizing a pair of primers or one primer alone. The probes were tested by dot-blot hybridization on HBV plasmid DNA, slot-blot hybridization on total cellular RNA of Alexander cells and Southern blot hybridization on cellular DNA of Alexander cells and HBV plasmid DNA. They were also tested by in situ hybridization (ISH) on HBV-positive biopsy liver tissue. A ratio of dig-dUTP:dTTP of 1:3 gave highest sensitivity in DNA hybridization. No loss of amplification efficiency and sensitivity was observed when the final concentration of dig-11-dUTP and dTTP was reduced to 20 [mu]M and 60 [mu]m respectively, compared to 200 [mu]m each of dATP, dCTP, dGTP. Several different sizes of double-strand probes were compared by dot-blot hybridization. Longer probes were more sensitive. Strong signal could also be obtained by combination of two or three small probes, which have overlapping sequences. Single-stranded DNA probes had advantages of simplicity of use, high sensitivity and strand specificity.


http://www.sciencedirect.com/science/article/B6WNC-49HSTV6-1/2/feb91b218644ec50acae01df1aa9a62e

Haplotype analysis using microsatellite markers is a useful indicator of specific mutations and is often exploited as the first large-scale screening technique to carry out the molecular characterization of the disease gene in probands from a specific population. However, the methodologies available are still cumbersome and require the use of either radioactive compounds or specialized equipment suitable to follow fluorescent dyes. Both these techniques may not be available for newly developing clinical laboratories. We have set up a sensitive and easy-to-use protocol to characterize five closely spaced, highly polymorphic microsatellite polymorphisms (CA repeats) that span the Wilson disease (WD) region, i.e. D13S316, D13S133, D13S301, D13S314, D13S315. The technique described here for the analysis of the WD gene microsatellite system relies on the quick detection method of silver staining, avoiding the use of toxic or sophisticated equipment. This approach could be the method of choice to implement molecular genetic testing in clinical laboratories, even those not especially equipped for DNA analysis and in particular in newly developed molecular genetics centers in countries whose
population has not yet been characterized for WD-causing ATP7B gene mutations.


http://www.sciencedirect.com/science/article/B6WNC-4CB0HNX-1/2/47788539570c45d133bf7f2ac2ed128a

A multiplex PCR for the simultaneous detection of some pathogenic genes of enteropathogenic, enterotoxigenic and verocytotoxin-producing Escherichia coli was developed. In this study primers found in literature as well as primers to the purpose designed were used. In this way, it was possible to generate specific fragments of 96, 170, 229, 285, 348, 414 and 510 bp for Hlya, St, EaeA, Lt, Vt1, UidA and Vt2 genes, respectively. When applied to bacterial strains experimentally inoculated in milk and milk products, the proposed PCR showed a detection limit of 5 x 10^4 CFU/ml for Hlya, St, EaeA, Vt1 primers, while for Lt and Vt2 primers the limit resulted of 10^6 CFU/ml.


http://www.sciencedirect.com/science/article/B6WNC-4DYN471-2F/2/331343dc3f5c815d3f106a7aedcd237c7

Ureaplasma urealyticum has been associated with a variety of disease conditions in humans. However, its exact etiologic role has not been well established because of the difficulties encountered in cultural diagnosis and the time needed for positive identifications. A DNA probe which is specific for a target DNA sequence unique to this suspected pathogen offers a rapid, sensitive and specific means of diagnosis. This study details the development of a polymerase chain reaction system for U. urealyticum. Using conventional hybridization techniques, a cloned genomic fragment was found to be specific for this organism. Sequencing of part of this probe DNA permitted the assignment of oligonucleotide primers which amplified a 186 bp target segment. This PCR system is specific for U. urealyticum but not for other closely related species of mycoplasma. This highly sensitive diagnostic technique will aid in determining the etiologic role, tissue tropism and dynamics of pathogenesis of this organism, and thereby result in better patient care.


http://www.sciencedirect.com/science/article/B6WNC-4938M0C-1/2/923b86c6e539907e236960ab9970126e

Salmonella infections continue to cause gastrointestinal and systemic disease throughout the world. Another concern with this pathogen is the ability to acquire integrons that confer resistance to multiple antibiotics. For multiresistant Salmonella enterica serotype Typhimurium, the most common multiresistant Salmonella serotype, an integron structure can be found between thdF and a retron. Our objective was to investigate the utility of a 450 bp thdF-retron amplicon as an
indicator of an insertless thdF-retron junction thus indicating an integron-free strain. Surprisingly, we found that the 450 bp thdF-retron amplicon was present, and thus incorrectly suggesting an integron-free status, in some multiresistant S. enterica serotype Typhimurium isolates. However, this phenomenon was not observed if the isolate was enriched in the presence of two antibiotics. This demonstrates that, within some individual clinical isolates of multiresistant S. enterica serotype Typhimurium, there exists a small subpopulation of integron-free bacteria. Consequently, it appears that the thdF-retron amplicon is an inaccurate predictor of integron status in S. enterica serotype Typhimurium unless multiresistance is used as a selection tool during enrichment.


http://www.sciencedirect.com/science/article/B6WNC-4CHRGDY-2/2/5f78e57c627066509a23c40245c79db5

A multiplex polymerase chain reaction (mPCR) was developed and optimized for the simultaneous detection and differentiation of avian reovirus (ARV), avian adenovirus group I (AAV-I), infectious bursal disease virus (IBDV), and chicken anemia virus (CAV). Four sets of specific oligonucleotide primers were used in this test for ARV, AAV-I, IBDV, and CAV. The mPCR DNA products were visualized by gel electrophoresis and consisted of fragments of 365 bp for IBDV, 421 bp for AAV-I, 532 bp for ARV, and 676 bp for CAV. The mPCR assay developed in this study was found to be sensitive and specific. Detection of PCR-amplified DNA products was 100 pg for both CAV and IBDV, and 10 pg for both ARV and AAV-I and this mPCR did not amplify nucleic acids from the other avian pathogens tested. The mPCR demonstrated similar sensitivity in tests using experimental fecal cloacal swab specimens that were spiked with ARV, AAV-I, IBDV, and CAV, and taken from specific pathogen free (SPF) chickens. This mPCR detected and differentiated various combinations of RNA/DNA templates from ARV, AAV-I, CAV, and IBDV without reduction of amplification from feces.


http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-2P/2/b5d61e28b1c93341d2dbe43abc6cc142

An assay is described in which 11 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene can be screened simultaneously. Six different exons of the CFTR gene are amplified in a single multiplex amplification. Biotinylated dUTP is incorporated into the different fragments during the amplification process. A sample of this mixture is then hybridized to 21 different poly-dT tailed oligonucleotide probes which are bound to a nylon membrane. In order to screen the different mutations in a single step hybridization, the length of the different oligonucleotides and the amount used in the assay were optimized. The detection is performed by binding avidin-alkaline phosphatase to the biotin, followed by a chemiluminescent reaction. By means of this fast and sensitive assay, about 85% of all the cystic fibrosis mutations in the Belgian population can be detected.

http://www.sciencedirect.com/science/article/B6WNC-47CJ5D5-5/2/1ab695694b42544a7b3da1e86006035b

The introduction of multiplex PCR techniques to clinical laboratories has provided a means to streamline assays and to produce multiple results with minimal effort. While this methodology is very beneficial, care must be taken to ensure that reactions are properly optimized to allow for maximum sensitivity. This study was conducted to determine whether the sensitivity of multiplex-real-time PCR assays could be improved by increasing the concentration of DNA polymerase within a reaction. Multiplex reactions were designed to simultaneously detect the human HLA-DQ gene and a sequence from the UL83 region of the CMV genome. Two real-time PCR systems, one utilizing AmpliTaq Gold DNA polymerase and the ABI 7700 Sequence Detection System, and one utilizing FastStart Taq DNA polymerase and the Roche LightCycler were tested. The results indicated that increasing the AmpliTaq Gold concentration from 0.050 to 0.10 U/µl and the FastStart Taq concentration from 0.1875 to 0.375 U/µl increased detection sensitivity from 5000 to 50 CMV copies per PCR reaction. In separate experiments, commercially prepared mastermixes were utilized for both real-time PCR platforms as per the manufacturer's suggestions or with the addition of supplemental DNA polymerase. In assays designed to detect 4 CMV genome copies per reaction, the addition of 2.5 U of AmpliTaq Gold to TaqMan Universal Mastermix increased the detection rate from 21 to 67%, and the addition of 5 U of FastStart Taq to FastStart DNA Master Hybridization Probes mastermix increased the detection rate from 17 to 56%. These results indicate that increasing the DNA polymerase concentration in multiplex real-time PCR reactions may be a simple way to optimize assay sensitivity.


Cystic fibrosis (CF) is a common genetic disorder in Caucasians, and in some populations 70% of cases are associated with a 3 base pair (bp) deletion ([Delta]F508) in the CFTR gene. We have implemented a fluorescence-based, multiplex allele-specific polymerase chain reaction (MASPCR) assay for deletion of the [Delta]F508 mutation. Different allele-specific fluorescently-tagged primers are used in the PCR reaction to distinguish between normal and [Delta]F508 alleles. Fluorescent PCR products are then visualized in a single lane on an agarose gel following electrophoresis combined with real-time multicolour fluorescence detection. The approach simplifies diagnosis of the most common mutation in the CFTR gene, and holds promise for a multiplex allele-specific, fluorescence-tagged gene amplification strategy for detection of additional CF mutations which may result in more cost-effective testing without increasing the risk of missed or erroneous diagnoses.


http://www.sciencedirect.com/science/article/B6WNC-4DYN471-
The technique of polymerase chain reaction (PCR) is potentially superior to existing methods for detecting rickettsial infections in ticks. For this reason, we developed assays for identifying rickettsial infections in ticks by PCR. Our assays amplified a 500 bp fragment from the gene encoding the rOmp B protein of Rickettsia rickettsii. The selected primers amplified fragments of the predicted size from all spotted fever group rickettsiae (R. Rickettsii, R. Parkeri, R. Conorii, R. sibirica) tested. No amplified products were detected when typhus group rickettsiae (R. Canada, R. Prowazekii, R. typhi) were assayed. Using techniques described in this study, we reliably amplified the predicted product from hemolymph, saliva and ground leg tissue samples from live, partially fed, infected ticks. Samples derived from infected ticks preserved in 70% ethanol also were suitable for amplification by PCR. Similar assays performed with infected ticks preserved in 5% buffered formalin seldom gave positive results.


Pyrosequencing (TM) technology is a bioluminometric DNA sequencing method that employs a cascade of four enzymes to deliver sequence signals. To date this technology has been limited to the sequencing of short stretches of DNA. As an improvement to this technique, we have introduced a bacterial group-specific, multiple sequencing primer approach that circumvents sequencing of less informative semi-conservative regions of the 16S rRNA gene. This new approach is suitable for challenging templates, improving sequence data quality, avoiding sequencing of non-specific amplification products, lessening sequencing time, and moreover, this strategy should open the way for many new applications in the future. The group-specific, multiple sequencing primers can be applied in the Sanger dideoxy sequencing method as well. In addition, we have improved the chemistry of the Pyrosequencing system enabling sequencing of longer stretches of DNA, which allows numerous new applications.


A triplex PCR assay was developed and evaluated for efficacy in detecting Campylobacter jejuni, Salmonella spp., and Escherichia coli O157:H7 in a variety of raw and ready-to-eat food products. Following a short enrichment period, artificially contaminated food samples were subjected to a triplex PCR assay, which incorporated published primers for each food pathogen, a protocol for sample collection, and a PCR procedure designed specifically for the assay. The selected primers amplified fragment sizes of 159 bp, 252 bp, and 360 bp for C. jejuni, E. coli O157:H7, and Salmonella spp., respectively. This assay provides specific and reliable results and allows for the cost-effective detection of all three bacterial pathogens in one reaction tube.


http://www.sciencedirect.com/science/article/B6WNC-4DYN471-1P/2/a825a684ec55eacfb930e247364e6369

We obtained cervical swabs from 397 women participating in a human papillomavirus (HPV) prevalence study. Samples were assayed for HPV infection using ViraPap expanded cocktail (detecting HPV types 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52 and 56), ViraType and PCR amplifications. Consensus primers from the L1 region were used with generic and type-specific oligonucleotide probes. Additionally, the generic amplifications were analysed with a novel restriction digest scheme. Samples positive by these methods were confirmed by PCR amplification using primers from the E5 region specific for HPV types 6, 16 and 18. The presence of human DNA in the samples was verified with amplification of the human KM-19 haplotyping primers. Our results confirm that the PCR reporter oligomer hybridization method is more sensitive than ViraPap/ViraType, but encompasses a narrower range of HPV types. This is particularly true of the higher number types in the expanded cocktail. The narrow range seems to occur as the result of the reporter oligomer used in hybridization, rather than the consensus amplimer pair used. Amplification of a broader range of HPVs is seen on gels or using the restriction digest as confirmation of HPV infection. Both ViraPap and PCR methods of detection gave about a 10% rate of uninterpretable results. PCR methods indicated about 1.7 times as many positives, while showing overall agreement of 77.6% with ViraPap. Agreement on types ranged from 67% to 100%. All methods indicated large fractions of untypable HPVs.


http://www.sciencedirect.com/science/article/B6WNC-4F0GR5B-1/2/cdbcf718a1fcd4b2757ad9df1074bb9

Development of rapid amplification assays for the detection and identification of biological threat agents has become a focus of diagnostic efforts in recent years. The use of real-time PCR assays as diagnostic tools depends upon two critical processes. First, nucleic acid purification must provide template that is both amplifiable and free of PCR inhibitors. Second, the assays themselves must be sensitive and specific for their nucleic acid targets. A differentiation must be made between results achieved due to the lack of target nucleic acid (true negatives) and those produced due to the inability to amplify target DNA (false negatives) so confidence in negative reactions is possible. False negatives can occur when inhibitors are present in the sample being tested, especially if clinical samples such as blood are analyzed. To address the problem of detecting inhibition in purified nucleic acids, an exogenous internal positive control (IPC) based on Taqman[trademark] chemistry was developed. A previously optimized assay was cloned and the primer and probe sites were mutated to produce novel sequences with no known homology to published sequence data. The IPC was sensitive to a variety of inhibitors, including hemoglobin, heparin, EDTA, hemic acids, and fulvic acid. It was also equally sensitive to inhibition when labeled with either 6FAM or ROX dyes. In addition, the IPC was successfully multiplexed with agent specific assays without any loss in their sensitivity. The designed IPC assay has proven to be an effective tool for monitoring inhibitors of PCR and builds confidence in negative results obtained with agent specific assays.

A rapid colorimetric assay for the detection of DNA from Plasmodium falciparum malaria is described, allowing direct sequencing of amplified fragments in the positive samples. The method is based on amplification by the polymerase chain reaction (PCR), with incorporation of biotin and a lac operator sequence in the amplified target DNA. The PCR product was immobilized on streptavidin-coupled magnetic beads, and detected by the specific binding of an Escherichia coli lac repressor [beta]-galactosidase fusion protein. Positive samples were subsequently treated with alkali to generate single stranded templates, which were used for solid phase genomic sequencing. As targets for amplification and sequencing we selected a region of the gene for the antigen Pf155/RESA and a region of the parasite dihydrofolate reductase gene (PfDHFR/TS). We show here that both of these gene targets can be used for specific detection of P. falciparum in patient blood samples. Genomic sequencing of five patient isolates revealed no variation in the Pf155/RESA gene fragment. In a comparison of this sequence with conserved protein domains, a marked similarity to the src homology region 3 was detected. A point mutation was found in the PfDHFR/TS gene fragment of one of the clinical samples, replacing Ser108 with Asn. This mutation has earlier been described in pyrimethamine and cycloguanil resistant strains of P. falciparum.


A fluorogenic 5' nuclease PCR assay was evaluated for its ability to specifically detect and differentiate DNA of two Orthopoxvirus species. A pair of consensus primers that target a DNA segment of the Orthopoxvirus haemagglutinin gene, and two oligonucleotide probes, each labelled with a different fluorescent reporter dye and the same quencher dye, were used in a single-tube assay. The assay is based on the 5'->3' nuclease activity of AmpliTaq DNA polymerase that cleaves a fluorescein-labelled hybridized probe. Probe cleavage generates specific fluorescent signals whose intensity can be quantified by fluorometry. After evaluating the effects of various annealing temperatures and probe concentrations and normalizing the emission intensities of the reporter dyes, it was possible to detect and differentiate monkeypox and vaccinia virus DNAs on the basis of a single-base polymorphism. The sensitivity of the 5' nuclease PCR assay is comparable to the sensitivity of ethidium bromide-stained gels, but the assay provides higher specificity and virtually eliminates the need for laborious post-PCR processing.


A method, referred to as cassette-ligation mediated polymerase chain reaction (PCR), has been developed to permit selective and specific amplification of cDNA sequence from total cellular RNA. This technique comprises (i) digestion of cDNA with multiple restriction enzymes, (ii) ligation of cleavage products to double-stranded DNA cassettes possessing a corresponding restriction site and (iii) amplification of cassette-ligated restriction fragments containing a short,
known sequence (but not all the other ligation products) by PCR using the specific and cassette primers; the specific primer is designed to prime synthesis from the known sequence of the cDNA whereas the cassette primer anneals to one strand of the cassette. Sequencing from the cassette primer provides information to design a new primer for the next walking step. The amplified cDNA fragments are often larger than the maximum DNA fragments (500-600 bp) that can be sequenced without the need of synthesizing internal sequencing primer. Each of such large cDNA fragments is dissected into smaller DNA fragments by repeating cassette-ligation mediated PCR exploiting different restriction sites and different sets of cassette primers. This dissection process reduces the number of specific primers to a minimum, thereby increasing the speed of sequencing and minimizing the overall cost. We have successfully applied this cDNA walking and sequencing by the cassette-ligation mediated PCR to the sequencing of an entire 6.5 kb genome segment of hantavirus strain B-1. The complete sequencing was achieved by four successive walking steps with 13 viral specific and three cassette primers, corresponding to more than 50% reduction in the number of primers necessary to sequence the cognate gene of other strains belonging to the same virus genus.


http://www.sciencedirect.com/science/article/B6WNC-4BYJXJ0-2/2/5a48181bf135895a9186bafe26c61c3f

In the present study, PCR-based single-strand conformation polymorphism (SSCP) analysis of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) was applied to the genetic characterisation of Dicyoecaulus from red deer from New Zealand and to its differentiation from species of lungworm from cattle and other hosts. Based on SSCP profiles, Dicyoecaulus individuals from red deer from different geographical localities in New Zealand could be readily distinguished from those representing other lungworms examined, irrespective of low-level sequence variability in the ITS-2 (0.4-2.6%) detectable among individuals. The ITS-2 of Dicyoecaulus from red deer differed in sequence by ~7-35% from congeners from other cervid hosts, demonstrating that this parasite is genetically distinct from other species of Dicyoecaulus for which ITS-2 sequence data are presently available. The results emphasize the need for a large-scale molecular systematic study of Dicyoecaulus specimens from various species of cervid and other ruminant hosts and the usefulness of mutation scanning for taxonomic, epidemiological and population genetic investigations.


http://www.sciencedirect.com/science/article/B6WNC-49HMTKW-1/2/d6f7bb5d3260c777aff20f41b8bac8f0

The effects of comprehensive LNA substitution in PCR primers for amplification of human genomic DNA targets are presented in this report. Previous research with LNA in other applications has shown interesting properties for molecular hybridization including enhanced specificity in allele-specific PCR. Here we systematically modified PCR primers and conditions for the human genomic DNA targets APOB and PAH, along with a [beta]-globin amplification control, to study whether the number and position of LNA residues improves or diminishes amplification sensitivity and specificity. It was observed that the design rules for LNA substitution in PCR primers are complex and depend upon number, position and sequence context. Technical advantages were seen when compared to DNA controls for the best LNA primer designs, which were typically one to a few centrally located LNA residues. LNA advantages include increased
maximum annealing temperature (Tmax) and increased signal with limiting primer or Taq DNA polymerase. Several well-characterized designs exhibited different efficiencies with different brands of hot-start enzymes. Many shorter LNA primers were found to be functional compared to same-length non-functional native DNA controls. These results show that LNA-substituted PCR primers have potential for use in difficult PCR techniques, such as multiplex amplification at higher Tmax, once firm LNA primer design rules are established.


http://www.sciencedirect.com/science/article/B6WNC-49V3GFV-1/2/afdf418a82f36e8ac784c0c9b3a6ca76

The aim of this study was to evaluate the use of Locked Nucleic Acids (LNA) probes in 5'-nuclease PCR, by comparison with Minor Groove Binder (MGB) probes routinely practiced in laboratories on ABI Prism 7700. The comparison was made using a collection of Staphylococcus aureus strains that have already been characterized by MGB 5'-nuclease PCR assays in a previous study [Mol Cell Probes, submitted for publication]. The sensitivity and specificity of 5'-nuclease PCR assays targeting the Staphylococcal enterotoxin genes sea to see were compared and showed that the LNA and MGB methods were equivalent. In conclusion, the LNA 5'-nuclease PCR assays developed in this work provide a specific and sensitive alternative to the well-established MGB 5'-nuclease PCR assays used for the rapid detection of bacterial pathogens genes on ABI Prism 7700.


http://www.sciencedirect.com/science/article/B6WNC-49H0RCT-1/2/479cd70c2a25bd6b2a3d69bb6af9b8

We describe the development of a strategy based on 5' nuclease multiplex PCR for the rapid detection of nine enterotoxin genes (sea, seb, sec, sed, see, seg, seh, sei, sej) of Staphylococcus aureus. The genotyping scheme consists in identifying these nine enterotoxin genes by three 5' nuclease Triplex-PCR assays. The strategy was evaluated using a collection of S. aureus reference strains previously examined with conventional PCR assays, and by testing previously characterized food S. aureus field strains. The 5' nuclease Triplex-PCR assays correctly detected the se genes in all the reference strains. In tests with field strains there was generally excellent agreement with the results obtained by conventional PCR, except for some strains harbouring variant se genes. The detection limits of the Triplex-PCR assays evaluated using fivefold dilution of recombinant plasmids for each se gene ranged from 16 to 2000 copies of target se genes in the PCR tube. The 5' nuclease Triplex-PCR assays developed are fast and specific, and provide a useful diagnostic tool for the detection and genotyping of se genes. The development of this method is an improvement that should facilitate epidemiological investigations of staphylococcal food poisoning outbreaks.

We have developed and clinically tested a rapid and largely automated procedure to detect mutations in the coding region of a gene of interest. Our method relies on the automated sequencing of the complete cDNA, followed by an advanced mutation search-and-verification routine using an integrated set of computer analysis tools. We have applied our automated procedure to the diagnosis of familial hypercholesterolemia (FH) in 52 unrelated FH families, by sequencing the whole cDNA coding region of the LDLR gene. Here we report the procedures and performance of our method in the identification of the most common types of LDLR mutations: short deletions or insertions and point mutations. Our method can provide a standard procedure for the 'overnight' unequivocal identification of mutations in those genetic diseases where several different mutations, none clearly prominent, may affect a given gene.


The authors have used a primer-engineered duplex polymerase chain reaction (PCR) -restriction fragment length polymorphism (RFLP) for the simultaneous detection of factor V Leiden and prothrombin G20210A mutations. The method involves the generation of HindIII RFLPs, and the restricted amplification products are analyzed by agarose gel electrophoresis in a single gel lane. This method is simple an inexpensive, and readily adaptable to the routine in a clinical molecular diagnostic laboratory other that our own.


Methods were developed for the detection of Legionella in environmental water sources, based upon the polymerase chain reaction (PCR) and gene probes. All species of Legionella, including all 15 serogroups of L. pneumophila tested, were detected by PCR amplification of a 104 bp DNA sequence that codes for a region of 5S rRNA followed by radiolabelled oligoprobe hybridization to an internal region of the amplified DNA. Strains of L. pneumophila (all serogroups) were specifically detected based upon amplification of a portion of the coding region of the macrophage infectivity potentiator (mip) gene. Pseudomonas spp, that exhibit antigenic cross-reactivity in serological detection methods did not produce positive signals in the PCR-gene probe method using Southern blot analyses. Single cell, single gene Legionella detection was achieved with the PCR--gene probe methods.

A DNA amplification procedure using heat stable Taq polymerase and the polymerase chain reaction is described for the detection of Pseudomonas aeruginosa in specimens from cystic fibrosis patients. A set of primers was selected on the basis of the nucleotide sequence of the algD gene encoding GDP mannose dehydrogenase, a major enzyme in the biosynthesis of alginate by P. aeruginosa. Using this set of primers in conjunction with the polymerase chain reaction, P. aeruginosa could be specifically detected, with a sensitivity approximating 10 bacteria, in sputum harbouring large numbers of other respiratory pathogens, including Staphylococcus aureus and Haemophilus influenzae. These results suggest that amplification of specific sequences within the algD gene by the polymerase chain reaction may provide a highly sensitive and specific tool for the detection of P. aeruginosa in the early stages of pulmonary colonization.


Amplification of nucleotide sequences within the invA gene of Salmonella typhimurium was evaluated as a means of detecting Salmonella. A collection of 630 strains of Salmonella comprising over 100 serovars, including the 20 most prevalent serovars isolated from animals and humans in Canada, was examined. Controls consisted of 142 non-Salmonella strains comprising 21 genera of bacteria. Cultures were screened by inoculating a single colony of bacteria directly into a polymerase chain reaction (PCR) mixture which contained a pair of primers specific for the invA gene. The specific PCR product was a 284 bp DNA fragment which was visualized in 2% agarose gels. With the exception of two S. litchfield and two S. senftenberg strains, all Salmonella strains were detected. In contrast, none of the non-Salmonella strains yielded the specific amplification product. Non-specific amplification of a few non-Salmonella strains resulted in a product that was distinctly different in size from the specific 284 bp product. Specificity of amplification was further confirmed by demonstration of hybridization of a 32P-labelled invA gene fragment only to the specific 284 bp product. The detection of 99.4% of Salmonella strains tested and the failure to specifically amplify DNA from non-Salmonella strains confirm that the invA gene contains sequences unique to Salmonella and demonstrate that this gene is a suitable PCR target, with potential diagnostic applications.


The polymerase chain reaction (PCR) was carried out with the highly conserved E. coli ribosomal RNA gene sequences 1376-1395 and 1521-1540. Using these primers and reaction conditions specified by the manufacturer(s), a 165 bp fragment was synthesized using Taq polymerase from three different sources in the absence of any added template. Restriction enzyme analysis suggests the source of this bacterial DNA is neither E. coli nor Thermus aquaticus. A variety of different methods to eliminate it such as treatment with DNase, restriction enzyme digestion, and CsCl2 density gradient centrifugation were unsuccessful. Since the bacteria in which the Taq polymerase is produced are not the source of the DNA, some step(s) in the purification or reagents added to the enzyme must be involved. Thus it is likely other biological products are similarly contaminated. Although the problem is easily dealt with by running a no-template control
and choosing other primers if a problem exists, it is important to recognize the potential for a false-positive result.


Animals and their by-products have been implicated as important sources of verocytotoxigenic Escherichia coli (VTEC) associated with disease in humans. VTEC comprise a wide range of serotypes and produce a variety of closely related verocytotoxins (VT). A pair of oligonucleotide primers, targeting conserved sequences found in VT1, VT2 and VTE genes, was used to develop a polymerase chain reaction (PCR) procedure to detect all types of VTEC. Supernatants of boiled broth cultures of VTEC (223 strains) isolated from ground beef, ground pork, raw milk, bovine faeces and porcine faeces; non-VTEC E. coli (72 strains); and other enteric and food bacteria (76 strains) were tested by PCR. The verocytotoxigenicity of these strains was verified by the Vero cell assay. All 223 VTEC isolates, comprising over 50 different serotypes, were detected by the PCR procedure. Shigella dysenteriae type 1 was the only other bacterium that was positive in this assay. As little as 1 pg of VTEC DNA and as few as 17 cfu of VTEC could be detected with this method. The results indicate that these primers detect VTEC over a wide range of serotypes. This method may be applicable as a screening procedure for the detection of VTEC in samples of foods and faeces.


Nucleic acid sequence-based amplification (NASBA) is a technique that has been previously shown to selectively mediate the detection of RNA in microbial cells. In a series of tests, nucleic acids were extracted from Salmonella enterica serotype Typhimurium and Mycobacterium avium subsp. paratuberculosis, and subjected to four enzymatic treatments prior to NASBA. These enzymatic treatments were DNase, RNase, S1 nuclease, and RNase/S1 nuclease. The results obtained were different for the two bacteria. With S. enterica serotype Typhimurium, RNase and RNase/S1 nuclease abolished the NASBA signal, as expected. But with M. avium subsp. paratuberculosis RNase, S1 nuclease, and RNase/S1 nuclease had no effect on the NASBA signal, whereas DNase treatment abolished it. This indicates that in the latter bacterium, NASBA can detect DNA, and demonstrates the necessity of verifying the nucleic acid origin of a NASBA signal if detection of RNA is objective.


We have investigated the PCR amplification technique of viral nucleic acids as an alternative
protocol for diagnosis and epidemiological studies of rabies virus. A primer set mapping in the nucleoprotein cistron allowed a specific and sensitive amplification of infected brain material, fulfilling the diagnosis requirements. One hundred samples checked by Southern or dot-blot analysis using both radioactive and non-radioactive probes showed identical results in parallel with routine techniques. For molecular epidemiological studies we selected another set of conserved primers flanking the highly evolutive pseudogene ([Psi] gene) region. This set was found to be efficient for all tested fixed rabies virus strains or wild rabies virus isolates as well as the rabies-related Mokola virus. We describe a progressive characterization of the strain that could be extended from rapid typing by a limited panel of restriction enzymes, to the ultimate identification of the nucleotide sequence by an original direct sequencing technique of amplified segments.


http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-2S/2/f33be0efa66bb597043a165b0d7b6480

Samples of peripheral blood lymphocytes from 105 different blood donors were investigated for the presence of human cytomegalovirus (HCMV) DNA using the polymerase chain reaction (PCR) with primers specific for the Pst / w fragment (IE region). Viral DNA sequences were detected in 53 samples, a fifth of which had been previously serotyped as HCMV negative. In the latter cases, Western blot analysis re-determined two out of three individuals that were resampled as seropositive. PCR could therefore be used to extend existing methods employed for the identification of HCMV infected blood samples prior to transfusion to individuals in high risk groups. In addition, the value of PCR as a diagnostic test was evaluated in a small pilot study by comparing the results obtained with urine samples from babies suffering congenital infection and from other high risk patients, with data obtained by isolation of infectious virus or through the detection of immediate early antigens in infected cultures. Data from this study indicated that PCR is at least as sensitive as the other methods used in HCMV diagnosis.


http://www.sciencedirect.com/science/article/B6WNC-4DBSV8W-1/2/9c12b833a03a8e8eb03c6843315499f7

Since the introduction of the polymerase chain reaction the presence of contaminating bacterial DNA in the Taq polymerase preparations has hampered the use of this technique in microbiology. Lately, this inconvenience has equally impeded gene quantification in the field of cell or gene therapy, where bacterial genes such as LacZ are often used as tags to detect vectors or cells after their injection in the recipient organism. Several means to overcome the DNA contamination of Taq Polymerase have been reported with variable degrees of decontamination efficiency and alteration of the PCR reaction. Here we propose two protocols to efficiently quantify DNA or RNA from the LacZ gene by real-time PCR using either decontamination by low concentrations of DNAse I prior to PCR amplification or a highly purified Taq Polymerase which is devoid of detectable contamination.
Scrapie is a transmissible spongiform encephalopathy (TSE) which affects sheep and goats. TSEs are characterised by the conversion of the cellular prion protein (PrPC) into the pathological form PrPSc. The occurrence of scrapie in sheep is influenced by polymorphisms in the PrP gene; in particular, three codons (136, 154 and 171) are important in conditioning the susceptibility/resistance of sheep to the disease, with the Val/Val136 Arg/Arg154 Gln/Gln171 genotype being the most susceptible and the Ala/Ala136 Arg/Arg154 Arg/Arg171, the most resistant one. The latter genotype seems to confer, in sheep, resistance to the oral infection with bovine spongiform encephalopathy, as well. The selection of genetically resistant sheep populations represents the basis of the recent strategies against ovine TSE in the European Union (EU). Herein, we describe a rapid and simple method, based on the primer extension technique, for PrP genotype determination at codons 136, 154 and 171. Intra-laboratory validation of the method showed accuracy levels comparable to those of sequencing analysis. Such method could be used for both the application of the EU policies requiring PrP genotype analysis in all ovine TSE cases, and the large-scale genotyping claimed by the implementation of breeding programmes for genetic resistance to TSE in sheep.

Myotonic dystrophy type 2 (DM2) is a dominant inherited disorder clinically similar to myotonic dystrophy type 1 (DM1) with a peculiar pattern of multisystemic phenotypic features. The mutation responsible for DM1 is a CTG repeat in the 3' UTR of the dystrophia myotonica protein kinase gene (DMPK) on chromosome 19q13.3, while DM2 is caused by an unstable CCTG expansion in intron 1 of the zinc finger protein 9 gene (ZNF9) on chromosome 3q21.3. Southern blotting analysis is the conventional test used to determine the size of the repeats in the molecular diagnosis of DM2. However, the large number of CCTG repeats and their somatic instability complicates this diagnostic protocol. In order to improve the DM2 test, we have recently characterised a single nucleotide polymorphism located in the first intron of the ZNF9 gene. This SNP consists in a C to A nucleotide change, which creates or disrupts an ApaI enzyme restriction site, easily detectable by PCR amplification followed by restriction analysis. We genotyped this SNP in 30 unrelated DM2 patients and 70 unrelated Italians healthy individuals. Our results show that this polymorphism is in linkage disequilibrium with the DM2 mutation.
predicted to be a member of the family of tRNA-guanine-transglycosylases for queuine biosynthesis by protein sequence similarity at the Zn-binding site (Locus Link #79691 at http://www.ncbi.nlm.gov/LocusLink/). FLJ12960 is immediately downstream of the Dopamine Receptor D3 (DRD3) gene. A Simple Tandem Repeat (STR) polymorphism was identified in intron 8 of the FLJ12960 gene. Primers designed to amplify the CA repeat detect 16 alleles from 121 to 151 base pairs, with a heterozygosity of 0.74.


http://www.sciencedirect.com/science/article/B6WNC-4DYN471-23/2/da3772fa139a8452c19708354977b63

DNA amplification assays such as the polymerase chain reaction are being developed for the amplification of small quantities of microbial nucleic acids. These assays offer the potential for a great deal of sensitivity. However, the high level of sensitivity increases the likelihood of cross-contamination of amplified products and the generation of false-positive reactions. In addition, substances in body fluids can inhibit the efficient performance of the amplification reactions. We have developed an assay format in which microbial nucleic acids are specifically bound to a solid phase surface. Contaminating DNA and enzyme inhibitors present in the sample are removed by washing prior to the performance of the amplification reaction. We could use this system to amplify and detect small amounts of HIV DNA diluted in whole blood. The assay system could distinguish target DNA from contaminating DNA fragments generated by prior amplification reactions.


http://www.sciencedirect.com/science/article/B6WNC-49HMTKW-3/2/b8acd04ed072ee4b691f52a73d753c57

Enterohemorrhagic Escherichia coli are harmful human pathogens capable of causing bloody diarrhea and vomiting. An important serotype commonly associated with human illness is the E. coli O157:H7 serotype. Unlike other real-time polymerase chain reaction (PCR) methods for identifying E. coli O157:H7, this study describes the development and optimization of a real-time PCR method targeting a conserved point mutation at +93 in the uidA (gusA) gene that is unique to O157:H7, distinguishing it from non-O157:H7 serotypes. A TET-labeled Minor Groove Binder (MGB) DNA probe was designed for use in a 5’ nuclease PCR assay. Using a panel of two E. coli O157:H7 strains, three E. coli non-O157:H7 strains, and one non-E. coli species, the assay was optimized for the specific detection of the E. coli O157:H7 strains. Optimal conditions were identified at high anneal/extend temperatures, low magnesium concentrations, and low probe concentrations, resulting in correct identification of E. coli O157:H7 and non-O157:H7 strains. The improved specificity of MGB probes for single base pair mismatches such as the +93 uidA mutation provides a novel approach towards rapid identification of E. coli O157:H7.

http://www.sciencedirect.com/science/article/B6T9P-3Y5FP3Y-2/2/3311dceaa2bbf5de1f5de54ef766e8c6

Molecular Cell (10)


http://www.sciencedirect.com/science/article/B6WSR-4194JJS-3/2/35de22fb5a1d917aa36f89256338f5fc

In Salmonella typhimurium, ahpC encodes subunit C of alkyl hydroperoxide reductase, an enzyme that reduces organic peroxides. Here, we asked if ahpC could protect cells from reactive nitrogen intermediates (RNI). Salmonella disrupted in ahpC became hypersusceptible to RNI. ahpC from either Mycobacterium tuberculosis or S. typhimurium fully complemented the defect. Unlike protection against cumene hydroperoxide, protection afforded by ahpc against RNI was independent of the reducing flavoprotein, AhpF. Mycobacterial ahpC protected human cells from necrosis and apoptosis caused by RNI delivered exogenously or produced endogenously by transfected nitric oxide synthase. Resistance to RNI appears to be a physiologic function of ahpC. ahpC is the most widely distributed gene known that protects cells directly from RNI, and provides an enzymatic defense against an element of antitubercular immunity.


http://www.sciencedirect.com/science/article/B6WSR-41BDB49-B/2/e8aba504658307793b2b1b05e719ce8f

Flagellin, the main protein of the bacterial flagella, elicits defence responses and alters growth in Arabidopsis seedlings. Previously, we identified the FLS1 locus, which confers flagellin insensitivity in Ws-0. To identify additional components involved in flagellin perception, we screened for flagellin insensitivity mutants in the flagellin-sensitive accession La-er. Here, we describe the identification of a new locus, FLS2, by a map-based strategy. The FLS2 gene is ubiquitously expressed and encodes a putative receptor kinase. FLS2 shares structural and functional homologies with known plant resistance genes and with components involved in the innate immune system of mammals and insects.

Hedengren, M., BengtAsling, et al. (1999). "Relish, a Central Factor in the Control of Humoral but Not
The NF-κB-like Relish gene is complex, with four transcripts that are all located within an intron of the Nmdmc gene. Using deletion mutants, we show that Relish is specifically required for the induction of the humoral immune response, including both antibacterial and antifungal peptides. As a result, the Relish mutants are very sensitive to infection. A single cell of E. cloacae is sufficient to kill a mutant fly, and the mutants show increased susceptibility to fungal infection. In contrast, the blood cell population, the hematopoietic organs, and the phagocytic, encapsulation, and melanization responses are normal. Our results illustrate the importance of the humoral response in Drosophila immunity and demonstrate that Relish plays a key role in this response.


Gene activation in higher eukaryotes is often under the control of regulatory elements quite distant from their target promoters. It is unclear how such long-range control is mediated. Here we show that a single determinant of the human growth hormone locus control region (hGH LCR) located 14.5 kb 5' to the hGH-N promoter has a critical, specific, and nonredundant role in facilitating promoter trans factor binding and activating hGH-N transcription. Significantly, this same determinant plays an essential role in establishing a 32 kb acetylated domain that encompasses the entire hGH LCR and the contiguous hGH-N promoter. These data support a model for long-range gene activation via LCR-mediated targeting and extensive spreading of core histone acetylation.


Congenital nephrotic syndrome of the Finnish type (NPHS1) is an autosomal-recessive disorder, characterized by massive proteinuria in utero and nephrosis at birth. In this study, the 150 kb critical region of NPHS1 was sequenced, revealing the presence of at least 11 genes, the structures of 5 of which were determined. Four different mutations segregating with the disease were found in one of the genes in NPHS1 patients. The NPHS1 gene product, termed nephrin, is a 1241-residue putative transmembrane protein of the immunoglobulin family of cell adhesion molecules, which by Northern and in situ hybridization was shown to be specifically expressed in renal glomeruli. The results demonstrate a crucial role for this protein in the development or function of the kidney filtration barrier.

The p160 coactivators bind to and potentiate transcriptional activation by nuclear receptors by recruiting secondary coactivators such as the histone acetyltransferases p300 and CBP and the protein methyltransferase CARM1. The function of the highly conserved N-terminal basic-helix-loop-helix/Per-Arnt-Sim (bHLH-PAS) domain of p160 coactivators is unknown. This region is required for coactivator synergy among p160, p300, and CARM1 coactivators. We identified a coactivator, coiled-coil coactivator (CoCoA), which binds to this domain and thereby enhances transcriptional activation by the estrogen receptor and other nuclear receptors. Endogenous CoCoA was found simultaneously with p160 coactivators on the promoter of an endogenous estrogen-responsive gene. Reduction of endogenous cellular CoCoA levels inhibited the estrogen-stimulated expression of transiently transfected and endogenous genes. Moreover, CoCoA cooperated synergistically with GRIP1, CARM1, and p300 to enhance ER-mediated transcription. Thus, the N-terminal region of p160 coactivators contains an additional activation domain which contributes to coactivator function by recruitment of CoCoA.


A minor class of pre-mRNA introns whose excision requires a spliceosome containing U11, U12, U4atac/U6atac, and U5 snRNPs has been identified in plants, insects, and vertebrates. We have characterized single loci that specify the U6atac and U12 snRNAs of Drosophila melanogaster. P element-mediated disruptions of the U6atac and U12 genes cause lethality during the third instar larval and embryonic stages, respectively, and are rescued by U6atac and U12 transgenes. The P element disruption of U6atac results in excision defects of U12-type introns from several transcripts including an alternative U12-dependent spliced isoform of prospero, a homeodomain protein required for CNS development. Thus, we demonstrate the requirement for the U12 spliceosome in the development of a metazoan organism.


We have systematically explored the in vivo occupancy of promoters and open reading frames by components of the RNA polymerase II transcription initiation and elongation apparatuses in yeast. RNA polymerase II, Mediator, and the general transcription factors (GTFs) were recruited to all promoters tested upon gene activation. RNA polymerase II, TFIIIS, Spt5, and, unexpectedly, the Paf1/Cdc73 complex, were found associated with open reading frames. The presence of the Paf1/Cdc73 complex on ORFs in vivo suggests a novel function for this complex in elongation. Elongator was not detected under any conditions tested, and further analysis revealed that the majority of elongator is cytoplasmic. These results suggest a revised model for transcription initiation and elongation apparatuses in living cells.

http://www.sciencedirect.com/science/article/B6WSR-4195G0J-G/2/8d63159299e7d06247845e7b21338474

Immunoglobulin (Ig) and T cell receptor (TCR) genes are assembled during lymphocyte maturation through site-specific V(D)J recombination events. Here we show that E2A proteins act in concert with RAG1 and RAG2 to activate Ig V[kappa]J but not Ig[lambda] V[lambda]1 rearrangement in an embryonic kidney cell line. In contrast, EBF, but not E2A, promotes V[lambda]1 rearrangement. Either E2A or EBF activate IgH DH4J recombination but not V(D)J rearrangement. The Ig coding joints are diverse, contain nucleotide deletions, and lack N nucleotide additions. Ig[kappa] VJ recombination requires the presence of the E2A transactivation domains. These observations indicate that in nonlymphoid cells a diverse Ig repertoire can be generated by the mere expression of the V(D)J recombinase and a transcriptional regulator.


http://www.sciencedirect.com/science/article/B6WSR-41FKPV5-M/2/072a12f9f9072bb19eb63a7bd829c06

STAT5 is activated in a broad spectrum of human hematologic malignancies. We addressed whether STAT5 activation is necessary for the myelo- and lymphoproliferative disease induced by TEL/JAK2 using a genetic approach. Whereas mice transplanted with bone marrow transduced with retrovirus expressing TEL/JAK2 develop a rapidly fatal myelo- and lymphoproliferative syndrome, reconstitution with bone marrow derived from Stat5ab-deficient mice expressing TEL/JAK2 did not induce disease. Disease induction in the Stat5a/b-deficient background was rescued with a bicistronic retrovirus encoding TEL/JAK2 and Stat5a. Furthermore, myeloproliferative disease was induced by reconstitution with bone marrow cells expressing a constitutively active mutant, Stat5a, or a single Stat5a target, murine oncostatin M (mOSt). These data define a critical role for Stat5a/b and mOSt in the pathogenesis of TEL/JAK2 disease.

Molecular Genetics and Metabolism (18)


http://www.sciencedirect.com/science/article/B6WNG-47X6S94-1/2/11fc286299737ac059bd2d52ba70de11

Ornithine transcarbamylase (OTC) deficiency, transmitted as an X-linked trait, is the most common disorder of the urea cycle. At least 3.5% out of more than 230 mutations consist of large
gene deletions, involving one or more exons. Only in 78% of OTC patients the diagnosis was confirmed on DNA level. We analysed OTC intragenic polymorphisms and haplotypes, in an attempt to contribute to the clarification of unresolved cases, in three populations (Czech, Portuguese, and Mozambican) and identified six novel nucleotide changes, all of them occurring with frequency higher than 12.5% in Europeans. Five of these polymorphisms occur with a significant frequency also in Africans. The number and frequency of haplotypes defined with the newly reported markers differ in individual populations.


http://www.sciencedirect.com/science/article/B6WNG-4F2B9SB-6/2/ec682aa4d56c22cf0f84f25a8296136cc

Hypophosphatasia is an inherited disorder caused by mutations in the bone alkaline phosphatase gene. We report here 11 new mutations responsible for hypophosphatasia. Four of them were deletions or insertions resulting in frameshift, two affected a donor splice site and five were missense mutations. Site-directed mutagenesis and transfection experiments of missense mutations showed that the mutations resulted in loss of most enzymatic activity, confirming the disease-causing role of these mutations. Analysis of the 3D model of tissue non-specific alkaline phosphatase showed that among the five missense mutations, one affected a residue in the crown domain and four affected residues located in the calcium-binding region. Alignment of the protein sequences of the calcium-binding region from 11 species showed that the four residues coordinating the calcium ion and the residues affected by the missense mutations described here are conserved in vertebrates. Together, our results confirm the functional role of the calcium site and suggest that its function is likely to be specific to vertebrates.


http://www.sciencedirect.com/science/article/B6WNG-4DJBR5K-1/2/e3a52f4a6ba5f0844e8da97cf4afe56

We investigated if eight SRY-negative 46,XX true hermaphrodites presented mutations in WNT-4, in blood leukocytes and/or gonadal tissue, as the cause of their disorder. We designed the sequences of the reverse primer of exon 1 and the primers of exons 2-5. Direct sequencing of all five exons demonstrated no mutant alleles in any of the patients. The possibility of the existence of causative mutations in the untranslated regions of WNT-4, or within introns cannot be ruled out.


http://www.sciencedirect.com/science/article/B6WNG-47902N4-7/2/abaaeda6ef5c006a0bf4bf2b04ff15de
Graves' disease (GD) is a complex autoimmune thyroid disorder with a strong genetic component. Genome-wide screens resolved several susceptibility loci that contribute to the development of GD. One of the susceptibility loci (GD-1 locus) was mapped on chromosome 14q31. However, a susceptibility gene located within the GD-1 locus remains undefined. Here we screen eighteen single nucleotide polymorphisms (SNPs), each is situated at a corresponding positional candidate gene, located within the GD-1 susceptibility locus on chromosome 14q23-q32, for predisposition to GD using the transmission disequilibrium test in 126 simplex Russian families affected with GD. Among SNPs tested, a significant preferential transmission of the Ala allele (41 transmissions vs. 17 nontransmissions, corrected P = 0.031) of the Thr92Ala SNP within the DIO2 gene, encoding type II iodothyronine deiodinase, from parents to affected children was found in a Russian family data set. The Thr92Ala SNP of the DIO2 gene and the D727E substitution of the thyrotropin receptor (TSHR) gene have been found to be in pair-wise linkage disequilibrium. The A92/E727 haplotype showed significant preferential transmission from parents to affected sibling (17 transmissions vs. 8 nontransmissions, P = 0.039) in simplex families. This suggests that the Thr92Ala variant of the DIO2 gene is associated or may be in linkage disequilibrium with a functional DIO2 polymorphism which involves in the development of GD in a Russian population.

Carnitine-acylcarnitine translocase (CAC) deficiency is a rare autosomal recessive disorder of long-chain fatty acid oxidation with a severe outcome. We report mutation analysis in a cohort of 12 patients. Twelve mutations were identified of which 9 have not been reported so far (G28C, D32N, R178Q, P230R, D231H, 179delG, 802delG, 69-70insTGTGC, and 609-1g > a). Altogether, including our results, 22 mutations of the CAC gene have been published to date in 23 patients demonstrating the allelic heterogeneity of CAC deficiency. DNA-based prenatal diagnosis was performed for the first time in pregnancies at risk for CAC deficiency. Two fetuses were affected and one pregnancy was terminated by family decision. Two other fetuses had normal genotype and five others were heterozygotes. All the offspring of these seven pregnancies are alive and apparently healthy.

The hph-1 ENU-mutant mouse provides a model of tetrahydrobiopterin deficiency for studying hyperphenylalaninaemia, dopa-response dystonia, and vascular dysfunction. We have successively localized the hph-1 mutation to a congenic interval of 1.6-2.8 Mb, containing the
GCH gene encoding GTP cyclohydrolase I (GTP-CH I). We used these data to establish a PCR method for genotyping wild type, hph-1 and heterozygote mice, and found that heterozygote animals have partial tetrahydrobiopterin deficiency. These new findings will extend the utility of the hph-1 mouse in studies of GTP-CH I deficiency.


http://www.sciencedirect.com/science/article/B6WNG-4C40SRM-4/2/84c7b0d2a2fb73b548d0e740ba5faa12

We describe the clinical findings, and the molecular and biochemical studies in an Italian family with recurrent hydrops fetalis due to galactosialidosis (GS). GS is a rare lysosomal storage disorder caused by a deficiency of the protective protein/cathepsin A (PPCA). This protein forms a high-molecular-weight complex with the hydrolases [beta]-galactosidase (GLB1) and neuraminidase (NEU1). By virtue of this association these two enzymes are correctly compartmentalized in lysosomes and protected against rapid proteolytic degradation. Controversial data show that PPCA is also present in a second complex, including the Elastin Binding Protein (EBP) the EBP-receptor, which is involved in elastogenesis, and NEU1. We investigated the potential role of the PPCA in both complexes. Two new genetic lesions (c60delG and IVS2 + 1 G > T) that lead to a frameshift and a premature stop codon were detected in the PPCA cDNA and genomic DNA of the patient. The deleterious effect of such mutations was confirmed by the complete absence of the PPCA protein on Western blots. Thus, we examined the effect of the loss of PPCA on the two protein complexes in the patient's fibroblasts. Interestingly, a reduced amount of both GLB1 and EBP proteins was detected. These data confirm that PPCA is present in two functional complexes one with GLB1 and NEU1 in the lysosomal lumen and the other with EBP at the cell surface. The reduction in GLB1 and EBP confirms that PPCA is essential for their integrity.


http://www.sciencedirect.com/science/article/B6WNG-46952MC-6/2/9cf0c83f085468b5440066f37ec4a89a


http://www.sciencedirect.com/science/article/B6WNG-4FB3X5V-4/2/0dfca261ace4cd5a5774bf0ae4c4a0ab

Globoid cell leukodystrophy (GLD, Krabbe disease) is a severe demyelinating disease caused by a genetic defect of [beta]-galactocerebrosidase (GALC). To date treatment to GLD is limited to hematopoietic stem cell transplantation. Experimental approaches by means of gene therapy in twitcher mouse, an authentic murine model of human GLD, showed significant but only marginal improvements of the disease. To clarify whether the introduction of GALC could provide beneficial effects on the oligodendrocytes in GLD, we transduced twitcher oligodendrocytes by
stereotactically injecting recombinant retrovirus encoding GALC-myc-tag fusion gene into the forebrain subventricular zone of neonatal twitcher mouse. In vivo effects of exogenous GALC on twitcher oligodendrocytes were studied histologically by combined immunostaining for the myc-epitope and the oligodendroglial specific marker, [pi] form of glutathione-S-transferase, at around 40 days of age. We show here that GALC transduction led to dramatic morphological improvement of the twitcher oligodendrocytes comparing with those in untreated twitcher controls. This study provided direct in vivo evidence that GALC transduction could prevent or correct aberrant morphology of oligodendrocytes in GLD which may be closely related to the dysfunction and/or degeneration of oligodendrocytes and the demyelination in this disease.


Hyperapobetalipoproteinemia is a common feature of the metabolic syndrome and could result from the interaction between genetic and dietary factors. The objective of this study was to verify whether dietary fat intake interacts with the T94A polymorphism of the liver fatty acid-binding protein (LFABP) gene to modulate plasma apolipoprotein (apo) B levels. Dietary fat and saturated fat intakes were obtained by a dietitian-administered food frequency questionnaire and the LFABP T94A genotype was determined by a PCR-RFLP based method in 623 French-Canadian men recruited through the Chicoutimi Lipid Clinic (279 T94/T94, 285 T94/A94, and 59 A94/A94). The LFABP T94A polymorphism was not associated with plasma apo B levels when fat intake was not taken into consideration. However, in a model including the polymorphism, fat intake expressed as a percentage of total energy intake, the interaction term and covariates, the variance in apo B concentrations was partly explained by the LFABP T94A polymorphism (5.24%, p=0.01) and by the LFABP T94A * fat interaction (6.25%, p=0.005). Results were similar when saturated fat replaced fat intake in the model (4.49%, p=0.02 for LFABP T94A and 6.43%, p=0.004 for the interaction). Moreover, in men consuming more than 30% of energy from fat, the odds ratio for having plasma apo B levels above 1.04 g/L for A94 carriers was of 0.40 (p=0.02) compared to T94/T94 homozygotes. Results were similar for carriers of the A94 allele consuming more than 10% of energy from saturated fat (OR: 0.32, p=0.005). In conclusion, T94/T94 exhibit higher apo B levels whereas carriers of the A94 allele seem to be protected against high apo B levels when consuming a high fat and saturated fat diet. These findings reinforce the importance to take into account gene-diet interactions in the prevention and management of the metabolic syndrome.


Nonketotic hyperglycinaemia (NKH) is an autosomal recessive disorder of glycine metabolism caused by a deficiency in the mitochondrial glycine cleavage enzyme. The majority of cases are caused by mutations in the P-protein, one of the four components of the glycine cleavage enzyme, also known as glycine decarboxylase (GLDC). Previous studies searching for causative mutations in NKH patients have only looked for a limited number of specific mutations or only screened part of the gene, and in many cases either no mutation or only one mutation was found, which is of limited use for prenatal diagnosis. In this study, we describe the screening of the entire
GLDC gene in 3 NKH families by D-HPLC analysis of all 25 exons, identifying two point mutations and two large deletions (exon 8 and exons 2-15) using a combination of D-HPLC analysis, long range PCR, Southern blot and sequencing. For complete prenatal testing both mutations need to be identified, and we suggest that screening of the entire gene as well as deletional analysis should be considered in those subjects where only one mutation has been identified.


http://www.sciencedirect.com/science/article/B6WNG-4CS4K95-1/2/d27a8b2257de41cb0e0eb2c16981cdbc

Mutations in sarcomeric proteins can lead to either hypertrophic or dilated cardiomyopathy depending on their effects on the structural and functional properties of the contractile unit of the heart. Mutations in cardiac troponin T, which binds the calcium-responsive troponin complex to [alpha]-tropomyosin, have been shown to result in cardiac hypertrophy or cardiac dilatation and heart failure, depending on the nature of the specific mutation. In this study, we report the identification of a novel cardiac troponin T mutation (A171S) leading to dilated cardiomyopathy and sudden cardiac death. In contrast to prior described mutations, the A171S mutation results in a significant gender difference in the severity of the observed phenotype with adult males (over 20 years of age) demonstrating more severe ventricular dilatation [left ventricular end diastolic dimension (LVEDD) 7.1 vs. 5.1 cm; P=0.01, t test] and left ventricular dysfunction [left ventricular shortening fraction (LVSF) 21 vs. 34%; P=0.04, t test] than adult females. The described mutation substitutes a hydrophilic amino acid for a hydrophobic one in a highly conserved domain involved in the interaction between troponin T and [alpha]-tropomyosin. Interestingly, four previously described mutations within 12 amino acids of A171 lead to a hypertrophic phenotype, suggesting that further characterization of the functional consequences of the A171S mutation may lead to a better understanding of the pathophysiology of DCM and of the functional differences between HCM- and DCM-causing mutations in cardiac troponin T.


http://www.sciencedirect.com/science/article/B6WNG-48PVDMC-1/2/b52fe98eac74d9e6b68aca10e6128e54

We identified a novel mutation in the glycogen phosphorylase gene (PGYL) in a Chinese patient with glycogen storage disease (GSD) type VI. The patient presented with gross hepatomegaly since the age of two without history of any hypoglycemic attack. Otherwise, he was largely asymptomatic. Liver tissue enzyme assays revealed a mild deficiency of total glycogen phosphorylase. Both PGYL and PHKA2 genes were sequenced. The patient was homozygous of a missense mutation G233D in PGYL. This location forms a hairpin turn secondary structure and the small glycine residue is completely conserved in all the orthologous proteins from Escherichia coli to mammals. This is the sixth reported mutation of this form of GSD.

We report 17 novel mutations that cause profound biotinidase deficiency. Six of the mutations are due to deletions, whereas the remaining 11 mutations are missense mutations located throughout the gene and encode amino acids that are conserved in mammals. Our results increase the total number of different mutations that cause biotinidase deficiency to 79. These additional mutations will undoubtedly be helpful in identifying structure/function relationships once the three-dimensional structure of biotinidase is determined.


Circulating levels of the cytokine interleukin 6 (IL-6) are elevated in obesity, correlate with body mass index (BMI), and predict the development of type 2 diabetes mellitus (T2DM). A promoter polymorphism in the IL6 gene is associated with obesity, altered levels of insulin sensitivity, and T2DM. IL-6 exerts its effects by binding to the IL-6 receptor (IL-6R) and levels of IL-6R have been correlated with BMI. It is possible that IL6R variants may also be related to obesity, but to our knowledge, no study has yet examined this relationship. The objective of this study was to examine the relationship between genetic variants in the IL6R gene and obesity in Pima Indians, a population prone to excess adiposity. We sequenced 6 kb of the IL6R gene, corresponding to all exons, exon-intron boundaries, and 2 kb of promoter in 30 Pima Indians. We identified six single nucleotide polymorphisms (SNPs) in the IL6R gene: a predicted Asp -> Ala substitution at position 358, a variant in the 3'-untranslated region, and 4 intronic SNPs. All SNPs were in strong linkage disequilibrium (D’[ges]0.90) and varied in minor allele frequency from 0.33 to 0.48. Association between IL6R genotype and BMI (kg/m2) was assessed in approximately 700 nondiabetic, full-heritage Pima Indians. For each SNP, individuals carrying the variant allele had a higher mean BMI compared to those with the wild-type allele (range: [37.3 +/- 7.2-38.2 +/- 7.0] vs. [35.5 +/- 7.3-36.0 +/- 7.5]; P=0.02-0.004). Our findings suggest that genetic variants in the IL6R gene may play a role in susceptibility to obesity. Assessment of these SNPs in other populations will be useful to determine the magnitude of obesity risk.

http://www.sciencedirect.com/science/article/B6WNG-47X1VRJ-5/2/124f377eb2b157a589e424654e002637

Linkage analysis has identified a susceptibility locus for type 2 diabetes mellitus (T2DM) on chromosome 1q21-q23 in several populations. Results from recent prospective studies indicate that increased levels of C-reactive protein (CRP), a marker of immune system activation, are predictive of diabetes, independent of adiposity. Because CRP is located on 1q21, we considered it a potential positional candidate gene for T2DM. We therefore evaluated CRP and the nearby serum amyloid P-component, APCS, which is structurally similar to CRP, as candidate diabetes susceptibility genes. Approximately 10.9 kb of the CRP-APCS locus was screened for polymorphisms using denaturing high performance liquid chromatography and direct sequencing. We identified 27 informative polymorphisms, including 26 single nucleotide polymorphisms (SNPs) and 1 insertion/deletion, which were divided into 7 linkage disequilibrium clusters. We
genotyped representative SNPs in ~1300 Pima samples and found a single variant in the CRP promoter (SNP 133552) that was associated with T2DM (P=0.014), as well as a common haplotype (CGCG) that was associated with both T2DM (P=0.029) and corrected insulin response, a surrogate measure of insulin secretion in non-diabetic subjects (P=0.050). Linkage analyses that adjusted for the effect of these polymorphisms indicated that they do not in themselves account for the observed linkage with T2DM on chromosome 1q. However, these findings suggest that variation within the CRP locus may play a role in diabetes susceptibility in Pima Indians.


Phospholipase A2, Group IVA (PLA2G4A) belongs to the class of cytosolic calcium-dependent phospholipases (cPLA2s) that preferentially cleave arachidonic acid (AA) from membrane glycerophospholipids. AA and AA metabolites play key roles in glucose disposal and insulin secretion. PLA2G4A is located on Chromosome 1q, where a number of groups have reported linkage to type 2 diabetes mellitus. We have screened the PLA2G4A gene and identified a C->G variant, which predicts a phenylalanine to leucine substitution. In logistic regression analyses adjusted for age, sex, ethnicity, and birth year, we found a trend toward association between this SNP and diabetes [OR=1.53 (0.97-2.40); p=0.06]. Individuals with the variant genotype had lower mean basal endogenous glucose output (1.8+/-0.03 vs. 1.9+/-0.01 mg/kg EMBS/min; p=0.04) and lower mean basal glucose oxidation (1.2+/-0.11 vs. 1.4+/-0.03 mg/kg EMBS/min; p=0.005) compared to individuals with the wild-type genotype. During a low dose insulin infusion, non-diabetic individuals with the variant genotype had a lower mean glucose oxidation (1.9+/-0.11 vs. 2.0+/-0.03 mg/kg EMBS/min; p=0.04) and total glucose turnover rate (2.5+/-0.22 vs. 2.6+/-0.06 mg/kg EMBS/min; p=0.01) compared to subjects with the wild-type genotype. In addition, under basal conditions, individuals with the variant genotype had a higher mean lipid oxidation rate compared to individuals with the wild-type genotype (0.77+/-0.25 vs. 0.67+/-0.23 mg/kg EMBS/min; p=0.02). These results provide evidence supporting a role for the eicosanoid biosynthesis pathway in type 2 diabetes mellitus pathophysiology.

http://www.sciencedirect.com/science/article/B6WNG-48JK1HJ-1/2/b4641a51943f060141a9e799314e2f44

Molecular Therapy  (11)


http://www.sciencedirect.com/science/article/B6WNJ-4CX0442-3/2/ad29328aed9b28823a6396b4b92a14e0

An SFG-based retroviral bicistronic vector containing a double-mutant dihydrofolate reductase-cytidine deaminase fusion cDNA (F/S DHFR-CD) with IRES-eGFP confers resistance to both methotrexate (MTX) and cytarabine (ara-C). Two weeks after transplantation with marrow
transduced with either a fusion or a control gene (eGFP-IRES-NeoR), human lymphoma (SKIDLCL-1) cells were injected sc into the flanks of nonobese diabetic/severe combined immune deficiency mice. In mock-transplanted mice, maximal tolerated dose (MTD) of posttransplant MTX/ara-C (15/10 mg/kg/day, x 3) was unable to control tumor growth. Transfer of the fusion gene allowed doses of MTX/ara-C (25/15 mg/kg/day, x 4) twofold higher than the MTD to be tolerated. The tumor burden defined the efficiency of posttransplant chemotherapy; early treatment, 48 h after tumor inoculation, provided tumor-free survival, while starting treatment after having palpable tumor growth (7 days) delayed tumor growth a median time of 28 days. In addition, the early treated group had higher gene expression in peripheral blood and marrow cells than the late treated group (P < 0.05), suggesting that early treatment allowed for enrichment of transduced marrow progenitors. These results encourage clinical studies using this retroviral fusion gene construct.

Kawamoto, K., M. Yagi, et al. (2003). "Hearing and hair cells are protected by adenoviral gene therapy with TGF-β1 and GDNF." Molecular Therapy 7(4): 484.

http://www.sciencedirect.com/science/article/B6WNJ-484SKC8-4/2/70e25f43022bd8276359de1ab799a6f5

Glial cell line-derived neurotrophic factor (GDNF) overexpression in the inner ear can protect hair cells against degeneration induced by aminoglycoside ototoxicity. The protective efficiency of GDNF increases when it is combined with co-factors such as transforming growth factor [β]1 (TGF-[β]1), a ubiquitous cytokine. The aim of this study was to determine whether TGF-[β]1 receptors are expressed in the inner ear and whether a cocktail of GDNF and TGF-[β]1 transgenes provides enhanced protection of the inner ear against ototoxic trauma. Using RT-PCR analysis, we determined that both TGF-[β]1 receptors, type 1 and 2 are present in rat cochlea. We co-inoculated two adenoviral vectors, one encoding human TGF-[β]1 gene (Ad.TGF-[β]1) and the other encoding human GDNF gene (Ad.GDNF) into guinea pig cochleae 4 days prior to injecting an ototoxic dose of aminoglycosides. Inoculated ears had better hearing and fewer missing inner hair cells after exposure to the aminoglycoside ototoxicity, as compared with controls and ears treated only with Ad.GDNF. Cochleae with TGF-[β]1 overexpression exhibited fibrosis in the scala tympani regardless of the presence of GDNF. Our results suggest that the adenovirus-mediated overexpression of GDNF and TGF-[β]1 can be used in combination to protect cochlear hair cells and hearing from ototoxic trauma.


http://www.sciencedirect.com/science/article/B6WNJ-458P5J1-5/2/5d90565fd191514ce68c084d8f68983

Real-time PCR is a powerful method for the quantification of gene expression in biological samples. This method uses TaqMan chemistry based on the 5'-exonuclease activity of the AmpliTaq Gold DNA polymerase which releases fluorescence from hybridized probes during synthesis of each new PCR product. Many gene therapy studies use lacZ, encoding Escherichia coli [β]-galactosidase, as a marker gene. Our results demonstrate that E. coli DNA contamination in AmpliTaq Gold polymerase interferes with TaqMan analysis of lacZ gene expression and decreases sensitivity of the method below the level required for biodistribution and long-term gene expression studies. In biodistribution analyses the contamination can lead to false-negative results by masking low-level lacZ expression in target and ectopic tissues, and false-positive results if sufficient controls are not used. We conclude that, to get reliable TaqMan results with lacZ, adequate controls should be included in each run to rule out contamination from
AmpliTaq Gold polymerase.


http://www.sciencedirect.com/science/article/B6WNJ-48S335N-3/2/914fd1b4e5e91a9aed457c3e3f924ea9

It is known that cellular proliferation, by either compensatory regeneration or direct hyperplasia, can augment lentiviral vector transduction into hepatocytes in vivo. For this reason, the present study was designed to determine if adolescent mice (31/2 weeks of age), which still have relatively proliferating livers, would have differential transduction compared to older (7 weeks of age) mice. Self-inactivating lentiviral vectors containing the human [alpha]1-antitrypsin (hAAT) promoter driving the expression of either the bacterial lacZ gene or the hAAT cDNA were generated for these studies. We found that adolescent mice given lentiviral vectors expressing lacZ (50 μg p24/mouse) via intravenous administration had a significantly higher level of hepatocyte transduction as measured by X-gal staining of liver sections compared to the 7-week-old mice. In addition, serum hAAT levels were nearly 40-fold higher in 31/2-week-old mice administered lentiviral vectors expressing hAAT (50 μg p24/mouse) compared to the 7-week-old mice. Moreover, the incorporation of a matrix attachment region from immunoglobulin [kappa] significantly increased transduction of hepatocytes in vivo. Although there was a small reduction in the circulating levels of hAAT, likely due to an immune response against the transgene product, gene expression was sustained for the duration of the study (30 weeks in total). In conclusion, the present study strongly demonstrates that lentiviral vector transduction efficiency and transgene expression were significantly enhanced in adolescent compared to older mice.


http://www.sciencedirect.com/science/article/B6WNJ-4D2MWN3-1/2/5b188e67d5ba1ad7fb85d946763844d5

Uncontrolled insertion of gene transfer vectors into the human genome is raising significant safety concerns for their clinical use. The wild-type adeno-associated virus (AAV) can insert its genome at a specific site in human chromosome 19 (AAVS1) through the activity of a specific replicase/integrase protein (Rep) binding both the AAVS1 and the viral inverted terminal repeats (ITRs). AAV-derived vectors, however, do not carry the rep gene and cannot maintain site-specific integration properties. We describe a novel hybrid vector carrying an integration cassette flanked by AAV ITRs and a tightly regulated, drug-inducible Rep expression cassette in the framework of a high-capacity, helper-dependent adenoviral (Ad) vector. Rep-dependent integration of ITR-flanked cassettes of intact size and function was obtained in human primary cells and cell lines in the absence of selection. The majority of integrations were site specific and occurred within a 1000-bp region of the AAVS1. Genome-wide sequencing of integration junctions indicates that nonspecific integrations occurred predominantly in intergenic regions. Site-specific integration was obtained also in vivo, in an AAVS1 transgenic mouse model: upon a single tail vein administration of a nontoxic dose of Ad/AAV vectors, AAVS1-specific integrations were detected and sequenced in DNA obtained from the liver of all animals in which Rep expression was induced by drug treatment. Nonrandom integration of double-stranded DNA can therefore be obtained ex vivo and in vivoby the use of hybrid Ad/AAV vectors, in the absence of toxicity and with efficiency compatible with gene therapy applications.
Liver is the most preferential site for metastasis of colon cancer. We, in the present study, constructed a self-replicable adenovirus in which E1A is driven by a CEA promoter and E1B-55K is deleted from the E1B region (AdCEAp/Rep) and examined its effects on multiple metastases of a human colon cancer cell in a mouse xenograft model. We first showed effective replication of the virus in various CEA-producing human colon cancer cells (M7609, HT-29) and subsequent lysis of the infected cells in vitro. We then demonstrated that a single intratumoral injection of the virus (1 X 10^8 PFU/100 [mu]l) induced a complete regression of subcutaneous tumors (M7609) inoculated into nude mice. Further, we demonstrated that systemic administration of the virus (1 X 10^8 PFU/100 [mu]l) through the tail vein to nude mice, which 1 week prior had been inoculated with tumor cells (colon carcinoma cell line HT-29) via the spleen and showed apparent multiple metastases in the liver, effectively suppressed the metastasis formation. The mean survival time of the treated mice was significantly longer than that of the controls. Thus, the systemic administration of AdCEAp/Rep was considered to be effective on multiple liver metastases of CEA-positive colon cancer in a xenograft model.

We have recently developed a replication-defective, recombinant adenovirus (Ad) vector composed of the whole Ad serotype 35 (Ad35), a member of subgroup B. We describe herein the in vitro and in vivo gene transfer properties of Ad35 vector in comparison with Ad serotype 5 (Ad5) and the Ad5F35 vector, which is a fiber-substituted Ad5 vector containing Ad35 fiber proteins. In vitro, Ad35 vector efficiently transduced not only human CAR-positive cells but also CAR-negative cells. Following intravenous administration into mice, both Ad5 and Ad35 vectors were rapidly cleared from the bloodstream with a half-life of approximately 3 min. Ad5 vector-mediated transgene expression predominantly occurred in liver parenchymal cells, although the Ad5 vector was delivered to both liver parenchymal and nonparenchymal cells. In contrast, Ad35 vector was efficiently taken up by liver nonparenchymal cells and mediated transduction efficiency in the liver on a level 4 log orders lower than the Ad5 vector. These findings demonstrate that Ad35 vector is an attractive vehicle for gene transfer into human cells, while the biodistribution profile of Ad35 vector in mice is much different from that of the Ad5 vector.

A principal concern regarding the safety of HIV-1-based vectors is replication-competent lentivirus.
We have developed two PCR assays for detecting RCL; the first detects recombination between gag regions in the transfer vector and the packaging construct (sensitivity of detection ~10-100 copies of target sequence). The second assay uses real-time PCR to detect vesicular stomatitis virus glycoprotein (VSVG) envelope DNA (sensitivity ~5-50 VSVG sequences). In an attempt to amplify any RCL, test vectors were used to transduce C8166 and 293 cells, which were then screened weekly for 3 weeks. Psi-gag recombinants were routinely detected (20 of 21 analyses) in four transductions using the RRL-CMV-GFP vector. In contrast, VSVG sequences were detected only once in 21 analyses. Interestingly, p24 levels (as measured by ELISA) were occasionally detectable after 3 weeks of culture. To determine if a true RCL was present, 21-day cell-free medium was used to transduce naive cells. No evidence of psi-gag or VSVG transfer was detected, indicating that the recombination events were insufficient to reconstitute a true RCL. These findings have important implications for the design and safety of HIV-1-based vectors intended for clinical applications.


http://www.sciencedirect.com/science/article/B6WNJ-4D4D25C-1/2/bb85481b77b0592b3330fa583a4f890

We produced lethally irradiated retrovirally GM-CSF-transduced autologous renal tumor cell vaccines (GVAX) from six Japanese patients with stage IV renal cell cancer (RCC). Four patients received GVAX ranging from 1.4 X 10^8 to 3.7 X 10^8 cells on 6-17 occasions. Throughout a total of 48 vaccinations, there were no severe adverse events. After vaccination, DTH skin tests became positive to autologous RCC (auto-RCC) in all patients. The vaccination sites showed significant infiltration by CD4+ T cells, eosinophils, and HLA-DR-positive cells. The kinetic analyses of cellular immune responses using peripheral blood lymphocytes revealed an enhanced proliferative response against auto-RCC in four patients, and cytotoxicity against auto-RCC was augmented in three patients. T cell receptor [beta]-chain analysis revealed oligoclonal expansion of T cells in the peripheral blood, skin biopsy specimens from DTH sites, and tumors. Western blot analysis demonstrated the induction of a humoral immune response against auto-RCC. Two of the four patients are currently alive 58 and 40 months after the initial vaccination with low-dose interleukin-2. Our results suggest that GVAX substantially enhanced the antitumor cellular and humoral immune responses, which might have contributed to the relatively long survival times of our patients in the present study.


http://www.sciencedirect.com/science/article/B6WNJ-4967F4P-2/2/b8015fc7290ec41a9c75bce0990aa49e

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure due to defective stem cell function. FA patients’ cells are hypersensitive to DNA cross-linking agents such as mitomycin C (MMC), exposure to which results in cytogenetic aberrations and cell death. To date Moloney murine leukemia virus vectors have been used in clinical gene therapy. Recently, third-generation lentiviral vectors based on the HIV-1 genome have been developed for efficient gene transfer to hematopoietic stem cells. We generated a self-inactivating lentiviral vector expressing the FA group A cDNA driven by the murine stem cell virus U3 LTR promoter and used the vector to transduce side-population (SP) cells isolated from bone marrow of Fanconi anemia group A (Fanca) knockout mice. One thousand transduced SP cells
reconstituted the bone marrow of sublethally irradiated Fanca recipient mice. Phenotype correction was demonstrated by stable hematopoiesis following MMC challenge. Using real-time PCR, one proviral vector DNA copy per cell was detected in all lineage-committed cells in the peripheral blood of both primary and secondary recipients. Our results suggest that the lentiviral vector transduces stem cells capable of self-renewal and long-term hematopoiesis in vivo and is potentially useful for clinical gene therapy of FA hematopoietic cells.


http://www.sciencedirect.com/science/article/B6WNJ-4CVX3HM-1/2/22b6bc4aa85b9cbc08e047206d7cf951

Cell-based therapies have potential widespread applications in clinical medicine, and methods for controlling the fate of transplanted cells are needed. We have previously described a means for directing the growth of genetically modified cells in vivo using a derivative of the thrombopoietin receptor, mpl, that is reversibly activated by a drug called a chemical inducer of dimerization (CID). Since Jak2 participates in signaling from a number of different cytokine receptors (including mpl), we tested whether direct activation of the JH1 domain of Jak2 would broaden the repertoire of hematopoietic lineages responsive to the CID. While the engineered Jak2 induced a significant rise in genetically modified red cells, as we have observed previously with mpl, it lacked mpl's ability to expand genetically modified platelets and failed to expand genetically modified granulocytes, B cells, or T cells. These findings identify a signaling molecule other than mpl that can function as a cell growth switch in vivo and demonstrate that signaling molecules used for in vivo selection need not be confined to receptors. The erythroid-restricted growth response suggests that CID-activated Jak2 may be well suited to gene therapy applications in sickle cell anemia or [beta]-thalassemia.

Mutagenesis (5)


http://mutage.oupjournals.org/cgi/content/abstract/18/3/299

We investigated TP53 mutation patterns in cancers of the esophagus and cardia of patients coming from Lower Normandy, a region situated in the highest incidence area in Europe. To screen tumor samples, we first used denaturing gradient gel electrophoresis (DGGE), a well-characterized technique which constituted our reference method. Then the results were compared with those obtained by denaturing high performance liquid chromatography (DHPLC), a recent and automatic screening technology. Analysis of the TP53 mutations profile showed that the detected alterations were mainly point mutations. Ninety-seven percent (33/34) of esophageal squamous cell carcinoma samples presented at least one mutation or polymorphism. The proportion of somatic, non-silent and sequence-confirmed mutations was 76% (26/34). The most common substitutions were G[-&gt;]A transitions, which could be related to nitrosamines, acetaldehyde or factors prone to producing mucosal irritation, like hot beverages. G[-&gt;]T
transversions, which were also frequently detected, could originate from benzo[a]pyrene in tobacco smoke. A[-&gt;T] transversions were not revealed in our series, which constitutes a discordance with mutational spectra already performed in north-western France. Concerning adenocarcinoma of the esophagus and cardia, the alteration frequency was 69% (11/16), with a majority of G[-&gt;A] transitions at CpG dinucleotides. They are probably related to endogenous process mediated by inflammatory diseases like gastro-esophageal reflux and Barrett's esophagus. The main advantage provided by DHPLC was its ease of application. However, the optimization steps turned out to be quite critical, especially for sequences with high melting temperatures embedded in lower melting temperature fragments. Considering only the common sequences analyzed by the two techniques, four of the 46 positive samples detected by DGGE were not revealed by DHPLC. This result stresses the limited sensitivity of DHPLC compared with DGGE under the conditions described in this study.


http://mutage.oupjournals.org/cgi/content/abstract/17/2/105

N-ethyl-N-nitrosourea (ENU) is a potent monofunctional ethylating agent that has been found to be mutagenic in a wide variety of organisms from viruses to mammalian germ cells. To elucidate the mutagenicity of ENU at the Tk+/- locus of mouse lymphoma cells and to confirm the ability of the mouse lymphoma assay (MLA) to detect both point mutations and large DNA alterations, Tk+/- L5178Y cells were exposed to different doses of ENU. Treatment of the cells with ENU resulted in a linear dose response with mutant frequencies of up to 16-fold over control. Evaluation of mutant clone size showed that 36% of the 100 {micro}g/ml ENU-induced clones (66% in control) were small colony mutants and 64% (34% in control) were large colony mutants. DNA isolated from mutants in the control culture and the 100 {micro}g/ml ENU treatment group was analyzed for loss of heterozygosity (LOH) using allele-specific PCR. The majority of the small colony mutants, both ENU-treated (97%) and spontaneous (91%), lost the Tk1b allele. The percentage of allele loss in ENU-induced large colony mutants was distinctly different from that of the control. Twenty-three percent of ENU-induced large colony mutants lost their Tk1b alleles, whereas 73% of the large colony mutants from the control culture lost the allele (P < 0.001). Overall, 50% of the Tk mutants from the 100 {micro}g/ml ENU-treated cultures (86% in control) showed LOH. Our data indicate that ENU is a potent mutagen in mouse lymphoma cells and that 100 {micro}g/ml ENU induces equal numbers of point mutations and chromosomal mutations. This study serves to verify that the MLA detects both point mutations and chromosomal mutations.


http://mutage.oupjournals.org/cgi/content/abstract/18/1/77

The genotoxicity and cytotoxicity of a Chinese medicinal herb, Tripterygium hypoglaucum (level) Hutch (THH), was investigated in human promyelocytic leukemia (HL-60) cells using the hypoxanthine-guanine phosphoribosyltransferase mutation assay. THH showed clear cytotoxicity and mutagenicity in HL-60 cells at concentrations between 6.7 and 20.0 mg/ml. When the mutants were characterized by techniques based on multiplex PCR, 46.6% of induced mutants were found to have deletions, whereas only 7.7% of spontaneous mutants showed deletions. The rest were not characterized, but were assumed to be mainly point mutations. Mapping of all
intragenic deletion breakpoints showed a random distribution of breakpoints in nine exons. Deletion of exon 1 appeared as the only whole gene deletion, while deletions of exon 7/8 and 9 often occurred concomitantly (71.4%). It is concluded that THH is mutagenic in HL-60 cells, predominantly inducing deletions. Since this herb is widely used as a traditional medicine, its genotoxicity should be assessed in vivo in treated humans.


http://mutage.oupjournals.org/cgi/content/abstract/19/3/215

The lacI mutant frequency and mutation spectrum were determined in the bone marrow and testes of B6C3F1 lacI transgenic mice exposed by inhalation to ethylene oxide (EO). Groups of male transgenic lacI B6C3F1 mice were exposed to 0, 25, 50, 100 or 200 p.p.m. EO for up to 48 weeks (6 h/day, 5 days/week) and were killed at 12, 24 or 48 weeks of EO exposure for determination of lacI mutant frequency. In the bone marrow, the lacI mutant frequency was significantly increased at the two highest exposure levels (100 and 200 p.p.m.) and at the 48 week exposure time point. The shape of the exposure-response curve for lacI mutant frequency in the bone marrow was non-linear. DNA sequence analysis of the bone marrow mutation spectrum revealed that only AT[-&gt;TA transversions occurred at an increased frequency in EO-exposed mice: 25.4% in EO-exposed mice for 48 weeks (200 p.p.m.) compared with 1.4% in air controls. In testes, the lacI mutant frequency was increased at a single exposure level of 200 p.p.m. for 24 weeks. At 48 weeks, the lacI mutant frequency in testes was significantly increased to an equal degree at 25, 50 and 100 p.p.m. EO but not at 200 p.p.m. Analysis of the testes mutation spectrum in air control mice and in mice exposed to 200 p.p.m. EO for 48 weeks revealed that no single mutational type occurred at an increased frequency. In the testes, there was a small increase across all mutational types that was sufficient to increase the overall lacI mutation frequency although not significant individually. The mutation spectrum in testes of EO-exposed mice also revealed that the increased lacI mutant frequency observed at 25 or 50 p.p.m. EO was not due to an increase in mutant siblings (clonality). These data demonstrate that inhalation exposure to EO for up to 48 weeks produces distinct mutagenic responses in bone marrow and testes.


http://mutage.oupjournals.org/cgi/content/abstract/19/1/67

We report, for the first time, mutations in the Alu repeat regions in the genome of kidney tumors induced by diethylstilbestrol in Syrian hamsters. Among the 66 loci amplified by 11 random primers, 28 loci exhibited insertions, deletions or losses or gains in intensity in the genome of kidney tumor tissues compared with normal kidney tissues from age-matched hamsters. Higher numbers of mutated Alu loci were observed in the tumors of old hamsters compared with young hamsters. In N-ethyl-N-nitrosourea- and diethylstilbestrol-treated hamsters deletion of a 0.59 kb locus amplified with primer OPC03 was observed in most of the female hamsters, but not in male hamsters. An insertion mutation of a 0.498 kb locus amplified with primer OPC03 was observed in 12 of 36 diethylstilbestrol-induced kidney tumors. The cloning and sequencing of the 0.498 kb locus amplified with primer OPC03 revealed that it had significant sequence similarity to the mouse RIKEN cDNA clone. These findings indicate that age, sex and co-exposure to N-ethyl-N-nitrosourea influence mutations in the Alu repeat sequences in the genome of diethylstilbestrol-
induced kidney tumors in Syrian hamsters. Structural alterations in Alu repeats in critical target genes may be involved in diethylstilbestrol-induced carcinogenesis.

**Mutation Research Genomics (9)**


http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-5/2/e07dfe956294f9c0abaa386b2beb8ac

The search for DNA sequence variations (DSV) is emphasized with genetic studies of a large number of multifactorial diseases. Saturation of regions of interest with diallelic polymorphisms will be an essential step to pinpoint, through association studies, predisposing genes. We have developed a solid-phase method based on the ability of mismatch binding protein MutS to recognize single nucleotide mismatches. This approach was applied to the study of 83 sequence-tagged sites (STSs) extracted from an eight centimorgans (cM) chromosome 21 region. One-third of tested STSs were found to be polymorphic leading to a frequency of one DSV every 822 base pairs (bp). Sequencing of analyzed STSs showed the high reliability of the MutS-based technology for mismatches up to 2 bp in DNA fragments ranging in size from 200 bp to 1 kilobase (kb). The entire assay which is performed in a solid-phase format without the need of electrophoresis or sequencing, will provide an efficient tool for new polymorphism detection.


http://www.sciencedirect.com/science/article/B6T2F-453BS2D-2/2/6a0ce9b51263125ba3168099e89b3fef

In this work, we explored the existence of genetic variants within the SEL1L transcriptional regulatory region by direct sequencing of the basal promoter. SEL1L is the human ortholog of the Caenorhabditis elegans gene sel-1, a negative regulator of LIN-12/NOTCH receptor proteins. To understand the relation in SEL1L transcription pattern observed in different epithelial cells, we analysed its promoter activity. We found it to be considerably higher only in pancreatic cells. We then looked for the presence of genetic variability within this region by sequencing the minimal promoter of 63 individuals (126 alleles); two new and associated polymorphic variants were found only in few lung carcinoma bearing patients. The functional effects of this polymorphism was analysed by transient transfection assay which resulted in a significant increase in the transcriptional activity of the gene.


http://www.sciencedirect.com/science/article/B6T2F-3V4J6S2-1/2/553b33ca561d521b0e225d038468c26b
Sequencing of a human DNA ligase I cDNA clone derived from HeLa cells revealed two unreported differences with the published sequence: a single base change and a three-base deletion. Both differences are in exon 6, and were analyzed by amplifying a segment containing exon 5, intron 6, and exon 6. The first finding was that intron 6 is approximately 2.6 kb in size, not the 1 kb reported in the literature. By sequence analysis of amplified segments, the single-base difference in exon 6 was shown to be polymorphic, with HeLa cells heterozygous for the A/C difference. Analysis of 60 unrelated individuals found a frequency of 0.5 for each allele. Primer extension reactions across the exon 5/exon 6 boundary were performed on cDNA obtained from HeLa cells and human thymus. The results show that the three-base deletion is due to a variation in splicing. For both HeLa and thymus, two-thirds of the transcripts are like the published cDNA sequence and one-third have the three-base deletion. Finally, sequencing of part of intron 6 revealed the presence of a complex GT repeat consisting of a 48-50 nucleotide polyuridine tract followed by a variable number of GT residues. This entire unit of polyuridine tract plus GTs is repeated three times. Detection of the repeated sequences required the development of specialized cloning and PCR conditions. Analysis of a pedigree showed that this complex repeat is polymorphic.


Microsatellite instability of DNA samples of 79 sporadic colon cancer patients were analyzed. These samples were also screened to search mutations in the repeat sequences in the gene for the type II receptor of transforming growth factor-[beta] (TGF-[beta] RII) using polymerase chain reaction (PCR), electrophoresis with urea gel, and PCR-single strand conformation polymorphism (PCR-SSCP) method. The incidence of microsatellite instability, defined as severe replication error phenotype (RER) with microsatellite alterations in more than three loci, was 6%. Deletion and insertion of an A residue in the (A)10 region, which cause frameshift mutation, were found in four samples and their incidence in the samples with microsatellite instability was 80%. A novel nucleotide substitution of T for G at 1918, which causes missense mutation of arginine to leucine at codon 528, was found in a sample with microsatellite instability. The mutation at 1918 was in highly conservative amino acid residue.


In this study, we show that direct mutational analysis of genomic DNA can be performed on single somatic cells extracted from a frozen, immunohistochemically stained tissue section using laser-assisted capture microscopy. Eighty-nine single tumor cells were separately dissected from one case of human basal cell cancer (BCC) and p53 mutations were analyzed by direct semi-automated sequencing of PCR fragments. Amplification was obtained for at least one of the two analyzed exons from approximately 50% of the single tumor cells. Identical p53 mutations were found in widely spread areas of the tumor, suggesting a clonal proliferation originating from one cell. Interestingly, comparison between results of immunohistochemistry and genetic analysis of the single cells revealed the same p53 mutations irrespective of the p53 immunoreactivity. We
propose that this approach has a great potential to allow investigation of genotypic differences in single cells and more specifically to resolve important and fundamental questions determining cancer heterogeneity.


http://www.sciencedirect.com/science/article/B6T2F-4292MHS-1/2/4928e4a17e7dd3ddea3f92929350212

The Homo sapiens major histocompatibility complex (MHC) class 1 chain related gene A (MICA) was scanned for novel single nucleotide polymorphisms (SNPs) using a panel of DNA samples from African-, Japanese- and Mexican-Americans. Overlapping primer-pairs were used to amplify products in the size range of 300 to 400 bp that were sequenced and scanned for SNPs using Phred, Phrap, Polycphred and Consed sequence analysis programs. A total of 16 SNPs were detected, six of which represent new variant nucleotides in the Homo sapiens MICA gene. Three of the variants also represent amino acid changes in the MICA protein. Differences among the three ethnic panels in the frequency of the variant nucleotides observed were inconsistent, but significant for seven of the SNPs detected. Though a small sample size, this study represents the first multi-population based analysis of the frequency and distribution of SNPs in the MICA gene, a locus that may be essential in the antigenic recognition by [gamma][delta] T cells.


http://www.sciencedirect.com/science/article/B6T2F-3T888C3-8/2/8dd3f7b06ab06aa14ff2cbe73c4de62

This study is part of an ongoing project at the National Institute of Standards and Technology (NiST) that generates a panel of DNA clones containing the most common mutations found in the human p53 tumor suppressor gene. This panel will be made available as a reference source for evaluation and testing for p53 mutations. Single strand conformation polymorphism (SSCP) analysis has found widespread acceptance as a tool for simply and rapidly screening for mutations, albeit with a detection rate that can be below 100%. We have begun to analyze mutations found in exon 7 of the p53 gene by SSCP using laser induced fluorescence capillary electrophoresis (LIF-CE). PCR fragments, containing single point mutations, were amplified from genomic DNA isolated from cell lines using primers labeled with two different fluorophores. This dual labeling approach allowed better traceability of mobility shifts as a function of the experimental conditions. While analyzing the clones H596, Colo320, Namalwa and wild type (reference samples) at different temperatures, ranging from 25 to 45[deg]C, it was observed that each mutation responded in a unique way to changes in temperature both in magnitude and direction of shifts relative to the wild type sample. In a blinded study, ten p53 exon 7 samples were matched automatically, using ABI PRISM Genotyper(R) software, against the four reference samples. From these 10 samples, six were correctly identified as containing one of the reference mutations, two corresponded to wild type, and two were correctly identified as non-reference mutations. This approach should prove helpful in the rapid screening of target sequences that are known to bear a limited number of mutations.
mutations in human tissues." Mutation Research/Mutation Research Genomics 406(2-4): 79.

http://www.sciencedirect.com/science/article/B6T2F-3X70SXM-5/2/26840eedec4ff7ec2497ece3ac779e55

Background and induced germline mutagenesis and other genotoxicity studies have been
hampered by the lack of a sufficiently sensitive technique for detecting mutations in a small
cluster of cells or a single cell in a tissue sample composed of millions of cells. The most frequent
type of genetic alteration is intragenic. The vast majority of oncogenic mutations in human and
mammalian cancer involves only single base substitutions. We have developed universally
applicable techniques that not only provide the necessary sensitivity and specificity for site
specific mutagenesis studies, but also identify the point mutation. The exponential amplification
procedures of polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been
combined with restriction endonuclease (RE) digestion to enable the selective enrichment and
detection of single base substitution mutations in human oncogenic loci at a sensitivity of one
mutant in more than 10^7 wild type alleles. These PCR/RE/LCR procedures have been
successfully designed and used for codons 12 and 248 of the Ha-ras and p53 genes,
respectively, both of which contain a natural MspI restriction endonuclease recognition sequence.
These procedures have also been adapted for the detection and identification of mutations in
oncogenic loci that do not contain a natural restriction endonuclease recognition sequence. Using
PCR techniques, a HphI site was incorporated into the codons 12/13 region of the human N-ras
gene, which was then used for the selective enrichment of mutants at this oncogenic locus.
These PCR/RE/LCR procedures for base substitution mutations in codon 12 of the N-ras gene
were found to have the sensitivity of detection of at least one mutant allele in the presence of the
DNA equivalent of 10^6 wild type cells. Only one peripheral blood leukocyte DNA specimen out of
nine normal individuals displayed an observable Ha-ras mutation that was present at frequency
between 10^-5 and 10^-6. These PCR/RE/LCR techniques for detecting and identifying base
substitution mutations are universally applicable to almost any locus or base site within the
human or animal genome. With the added advantage of the adjustability of both the amount of
DNA (number of genomes) to be tested and the sensitivity (10^-2 to 10^-7) of the assay selection or
enrichment procedures, these PCR/RE/LCR techniques will be useful in addressing a broad
range of important questions in mutagenesis and carcinogenesis.


http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-3/2/cbadf6bf2b8ba92a9140c7999525dd27

We studied the DNA sequence of the entire coding region of ERCC1 gene, in five cell lines
established from human ovarian cancer (A2780, A2780/CP70, MCAS, OVCAR-3, SK-OV-3), 29
human ovarian cancer tumor tissue specimens, one human T-lymphocyte cell line (H9), and non-
malignant human ovary tissue (NHO). Samples were assayed by PCR-SSCP and DNA sequence
analyses. A silent mutation at codon 118 (site for restriction endonuclease MaelI) in exon 4 of the
gene was detected in MCAS, OVCAR-3 and SK-OV-3 cells, and NHO. This mutation was a C->T
transition, that codes for the same amino acid: asparagine. This transition converts a common
codon usage (AAC) to an infrequent codon usage (AAT), whereas frequency of use is reduced
two-fold. This base change was associated with a detectable band shift on SSCP analysis. For
the 29 ovarian cancer specimens, the same base change was observed in 15 tumor samples and
was associated with the same band shift in exon 4. Cells and tumor tissue specimens that did not
contain the C->T transition, did not show the band shift in exon 4. Our data suggest that this
alteration at codon 118 within the ERCC1 gene, may exist in platinum-sensitive and platinum-
resistant ovarian cancer tissues.

http://www.sciencedirect.com/science/article/B73H4-47P83MR-1C/2/7093b5820807d2b8beb8404809ed7b4

Human T-lymphocytes have been treated with benzo[a]pyrene diolepoxide (BPDE) in vitro and T-cell clones mutated in the hprt gene have been isolated. The mutant frequencies in BPDE-treated T-cell cultures were on average 24-fold higher than those of untreated cultures. Thus, BPDE is a potent inducer of gene mutation in this system. In order to examine which types of mutations are induced by BPDE in human cells, 41 spontaneous and 44 BPDE-induced mutant clones have been characterized using the Southern blot technique. In addition, rearrangements of the T-cell-receptor[beta] and [gamma] loci have been used to determine the proportion of isolated clones that are unique, and thus likely to represent independent mutational events. Out of 23 independent spontaneous mutants 4 had large hprt alterations that could be detected on Southern blots. Two of these alterations, deletions of exons 2-6, have been confirmed using PCR of hprt cDNA and direct sequencing of the PCR product. All 33 independent BPDE-induced mutants had normal hprt restriction patterns which indicates that BPDE is mainly a point mutagen in this system.


http://www.sciencedirect.com/science/article/B73H4-47PP1FH-9W/2/f42c09c4c7ba5f17678cbb778f66b7c4

A cloning assay was used to recover hprt- T-lymphocytes from adult human males. Analysis of crude cellular extracts by polymerase chain reactions (PCRs) demonstrated that 7% (16/218)of the hprt mutation were due to total deletion of the hprt gene. 14 of the 16 mutants were examined by PCR for the presence of flanking DNA to determine the extent of the deletions. The deletion mutation in 13 mutants was at least 350 kb with 5 of these deletions being at least 700 kb. The largest deletions were greater than 15 times the size of the hprt gene. Therefore, large deletions are tolerated at the hprt locus of the T-lymphocytes.


http://www.sciencedirect.com/science/article/B73H4-47PP0XS-3H/2/8a72fe18176c6ba63e5a56dd46894bce

Denaturing gradient gel electrophoresis (DGGE) is increasingly being utilized in mutational detection, both in characterization of variations in genomic DNA and in the generation of
mutational spectra after in vitro and in vivo mutagenesis. The basis for this electrophoretic separation technique is strand dissociation of DNA fragments in discrete, sequence-dependent melting domains followed by an abrupt decrease in mobility. We have modified the DGGE by using constant denaturant gels corresponding to the specific melting domains of certain DNA fragments. This leads to increased resolution of mutants as fragments differing in as little as 1 base pair migrate with a consistently different mobility through the whole gel allowing separations of several centimeters. By using a set of constant denaturant gels it is also possible to obtain a better approximation of the location of the different mutations as each denaturant concentration will correspond to specific melting domains. We have used this technique to separate 6 out of 7 exon-3 hypoxanthine phosphoribosyltransferase (HPRT) mutants while using conventional DGGE we were only able to separate 3.


http://www.sciencedirect.com/science/article/B73H4-47PP1FH-9X/2/4aba9503df047fc693eb79fb5d2a081

Adult male fish of the medaka HNI strain exposed to 9.5 Gy or 19 Gy (0.95 Gy/min) of [gamma]-rays were mated with non-irradiated female fish of the Hd-rR strain. Genomic DNA was prepared from malformed individual embryos which were expected to be dominant lethal and used for AP-PCR fingerprinting. By the use of a part of the T3 promoter sequence (20 mer), which to our knowledge, is not found in the medaka genome as an arbitrary primer, we found polymorphisms in genomic fingerprints which could distinguish the parental strains. On the other hand, we found that the fingerprints of F1 hybrids were the sum of those of their parents. Based on those findings, we analyzed the fingerprints of genomic DNA of each severely malformed embryo, because we expect that radiation-induced genomic damages resulting in severe malformation and eventually in dominant lethals should be detected as changes in paternal fingerprints of F1 hybrids. Indeed, we succeeded in detecting changes in genomic DNA as loss of some paternal bands in fingerprints of malformed embryos. One of 10 malformed embryos obtained from 9.5 Gy [gamma]-irradiated males had lost one band of the paternal origin and 4 of 12 malformed embryos obtained from 19 Gy [gamma]-irradiated males had lost 5 bands. These results indicated a possibility that quantitative as well as qualitative estimation of [gamma]-ray-induced DNA damages can be made by this method which does not require the functional selection based on a specific target gene.


http://www.sciencedirect.com/science/article/B73H4-47S0RVY-5/2/9707d6793c146e7eb62c63362a3ed1ef

Transgenic mouse models are being used with increasing frequency for mutational and toxicological studies. One such system, MutaMouse, contains a stably integrated lambda-gt10LacZ shuttle vector in the mouse genome. We describe the use of dual color fluorescence in situ hybridization (FISH) with Mus musculus whole chromosome paints and lambda DNA to map the integration site of the lambda transgene to band C on mouse chromosome 3.
The cDNA sequence of the Chinese hamster xeroderma pigmentosum group D (CXPD) nucleotide excision repair gene was analyzed from three Chinese hamster ovary (CHO) cell lines: repair proficient strain AA8 and repair deficient, UV complementation group 2 strains UV5 and UVL-13. CXPD encodes a presumed ATP-dependent DNA helicase and is single copy in CHO lines due to the hemizygosity of chromosome 9. Comparison of the deduced wild-type AA8 CXPD protein sequence with that of the Chinese hamster V79 lung-derived cell line revealed two amino acid polymorphisms. Position 285 is glutamine in AA8 and arginine in V79, and position 298 is alanine in AA8 and threonine in V79. Comparison with the human XPD, Saccharomyces cerevisiae RAD3, and Schizosaccharomyces pombe rad15 homologs shows variability at these positions. Analysis of the CXPD sequence in the repair deficient CHO lines UV5 and UVL-13 revealed, in each case, a single base substitution resulting in an amino acid substitution. Position 116 is tyrosine in UV5 and cysteine in AA8, and the corresponding positions of XPD, RAD3, and rad15 are cysteine. Position 615 is glutamic acid in UVL-13 and glycine in AA8, and the corresponding positions of XPD, RAD3, and rad15 are glycine. In both UV5 and UVL-13, positions 285 and 298 are glutamine and alanine, respectively, as seen in AA8. These results suggest that cysteine 116 and glycine 615 are critical to the repair function of CXPD.

Adriamycin (ADR), a commonly used cancer chemotherapy antibiotic, exhibits a variety of genotoxicities. In this study, we have examined the mutagenicity of ADR at the hypoxanthine-guanine phosphoribosyltransferase gene (hprt) in Chinese hamster ovary (CHO) cells and the xanthine-guanine phosphoribosyltransferase locus (gpt) in a pSV2gpt-transformed CHO cell line, AS52. Although ADR induced a dose-dependent increase of mutant frequency at both loci, it was more mutagenic to the gpt gene than to the hprt locus. Multiplex PCR analysis revealed that 35% of the 103 independent ADR-induced HPRT-deficient mutants carried large deletions. Among these deletion mutants, 33% were total gene deletions, 22% affected multiple exons, and 42% involved a single exon, of which most (9/15) were exon 1. The majority (63%) of ADR-induced AS52 mutants had a total deletion of the gpt gene. These observations indicate that ADR induces large deletions as a major type of gene mutation in mammalian cells, suggesting the involvement of reactive oxygen species as one mutagenic pathway in the mutagenesis of ADR.
Botryosphaeria stevensii frequently has been associated with dieback and canker diseases of oak, mainly in the western Mediterranean area but more rarely in other regions. The species concept of B. stevensii has been unclear, and it is possible that some collections were identified incorrectly. A collection of fungal strains isolated from diseased oak trees and initially identified as B. stevensii was characterized on the basis of morphology and ITS nucleotide sequences. Morphology was compared with the type specimens of Physalospora mutila (= B. stevensii) and its anamorph, Diplodia mutila. It was concluded that the isolates from oak differed from B. stevensii in having larger ascospores and conidia as well as different spore shapes and represented an as yet undescribed species, which is described here as B. corticola. Moreover, ITS sequence data separated B. corticola from all other known species of Botryosphaeria. Amended descriptions of B. stevensii and its anamorph are provided to differentiate B. stevensii from B. corticola and to clarify some of the earlier taxonomic uncertainties.


The phylogeny of selected gasteromycetes and hymenomycetes was inferred from partial nuclear large subunit rDNA (nuc-lsu, 28S) sequences, delimited by primers LR0R and LR5. Taxon sampling with emphasis on relationships within the Boletales further included some gasteroid groups, which obviously have evolved convergent fruiting body morphology, and therefore remained controversial in taxonomy. This study confirms the close relationship of Geastrales, Gauteriales and Phallales and the presumable derivation of Nidulariales and Tulostomatales within the euagarics clade, as widely accepted. In addition, four Hymenogaster species investigated were found to be in the euagarics clade and a relationship to the Cortinariaceae was indicated. The gasteroid fungus Zelleromyces stephensii is an example for maintaining morphological linkage by a lactiferous hyphal system to the genus Lactarius in the Russulales, and this relationship was affirmed in the sequence analysis. Several previously suggested relationships of gasteromycetes and Boletales were reproducible by analyzing nuc-lsu sequences. As a new result, Astraeus hygrometricus, the barometer earth star, is an additional representative of the Boletales. Together with Boletinellus, Phlebopus, Pisolithus, Calostoma, Gyroporus, Scleroderma, and Veligaster, Astraeus forms an unusual group comprising pileate-stipitate hymenomycetes and polymorphic gasteromycetes. This group is a major lineage within the Boletales and we propose the new suborder Sclerodermatineae, including the six families Boletinellaceae fam. nov. (Boletinellus and Phlebopus), Gyroporaceae (Singer) fam. nov. (Gyroporus), Pisolithaceae (Pisolithus), Astrapaceae (Astraeus), Calostomataceae (Calostoma), and the typus subordinis Sclerodermataceae (Scleroderma and Veligaster). Morphological and ecological characters, and pigment synthesis support the delimitation of the Sclerodermatineae, and indicate the radiation of different lineages in the Boletales originating from fungi with primitive tubular hymenophores. We regard such boletes with gyroid-boletoid hymenophores, like Boletinellus, Gyrodon, Gyroporus, Paragyrodon and Phlebopus as key taxa in the evolution of Paxillineae, Sclerodermatineae and Boletineae.

Neonectria (Hypocreales: Nectriaceae) species having Cylindrocarpon anamorphs that lack microconidia and chlamydospores include: Neo. discophora var. discophora, Neo. discophora var. rubi, stat nov. et comb. nov., Neo. lucida, comb. nov., Neo. viridispora, sp. nov. and Neo. westlandica, comb. nov. Perithecia of these species are red and perithecial anatomy is of the N. mammoidea type, with a palisade of hypha-like cells in the outer perithecial wall. These species occur on recently dead or dying trees. Perithecia of Neo. betulae, sp. nov and Neo. dumontii, sp. nov. are anatomically and biologically similar to those of Neo. discophora. The only known culture of Neo. betulae remained sterile, while Neo. dumontii has not been cultured; their anamorphs are presumed to be Cylindrocarpon. Analyses of mit ssu rDNA sequences indicate that Neonectria/Cylindrocarpon is monophyletic. Within the genus, species having N. mammoidea type perithecia are paraphyletic. Most species cluster with Neo. discophora, but Neo. westlandica and Neo. trachosa are basal to a clade that includes species that do not have a N. mammoidea-type perithecium. Nectria fuckeliana clusters independently of Neonectria and Nectria. Although reported to have a Cylindrocarpon anamorph, fresh ascospore isolates of N. fuckeliana did not produce Cylindrocarpon macroconidia but produced acremonium- or verticillium-like anamorphs. A key to nectriaceous species of Neonectria that have Cylindrocarpon anamorphs that lack microconidia and chlamydospores and/or that have a N. mammoidea type perithecial wall anatomy is presented. New combinations are proposed for other species formerly included in Nectria that have non-microconidial Cylindrocarpon anamorphs: Neonectria cinnamomea, Neo. jungneri, Neo. platycephala, Neo. phaeodisca and Neo. verrucospora.


http://www.mycologia.org/cgi/content/abstract/94/6/1017

The ascomycete order Diaporthales includes a number of plant pathogenic fungi such as Cryphonectria parasitica, the chestnut blight fungus, as well as many asexually reproducing fungi without known sexual states. Relationships among genera in the Diaporthales were evaluated as a basis for the recognition of families and to provide a taxonomic framework for the asexually reproducing diaporthalean fungi. Phylogenetic relationships were determined based on analyses of large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequences. Within the Diaporthales 82 sequences representing 69 taxa were analyzed. Results suggest the presence of at least six major lineages within the Diaporthales recognized as the Gnomoniaceae sensu stricto, Melanconidaceae sensu stricto, Schizoparme complex including the anamorph genera Coniella and Pilidiella, Cryphonectria-Endothia complex, Valsaceae sensu stricto, and Diaporthaceae sensu stricto. In addition, six teleomorphic and anamorphic taxa fell within the Diaporthales but were not allied with any of the six lineages.


http://www.mycologia.org/cgi/content/abstract/94/3/437

Anastomosis group 3 (AG-3) of Rhizoctonia solani (teleomorph = Thanatephorus cucumeris) is frequently associated with diseases of potato (AG-3 PT) and tobacco (AG-3 TB). Although isolates of R. solani AG-3 from these two Solanaceous hosts are somatically related based on anastomosis reaction and taxonomically related based on fatty acid, isozyme and DNA characters, considerable differences are evident in their biology, ecology, and epidemiology. However, genetic diversity among field populations of R. solani AG-3 PT and TB has not been documented. In this study, the genetic diversity of field populations of R. solani AG-3 PT and AG-3 TB in North Carolina was examined using somatic compatibility and amplified fragment length
polymorphism (AFLP) criteria. A sample of 32 isolates from potato and 36 isolates from tobacco were paired in all possible combinations on PDA plus activated charcoal and examined for their resulting somatic interactions. Twenty-eight and eight distinct somatic compatibility groups (SCG) were identified in the AG-3 PT and AG-3 TB samples, respectively. AFLP analyses indicated that each of the 32 AG-3 PT isolates had a distinct AFLP phenotype, whereas 28 AFLP phenotypes were found among the 36 isolates of AG-3 TB. None of the AG-3 PT isolates were somatically compatible or shared a common AFLP phenotype with any AG-3 TB isolate. Clones (i.e., cases where two or more isolates were somatically compatible and shared the same AFLP phenotype) were identified only in the AG-3 TB population. Four clones from tobacco represented 22% of the total population. All eight SCG from tobacco were associated with more than one AFLP phenotype. Compatible somatic interactions between AG-3 PT isolates occurred only between certain isolates from the same field (two isolates in each of four different fields), and when this occurred AFLP phenotypes were similar but not identical.


http://www.mycologia.org/cgi/content/abstract/94/3/450

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was developed to identify and differentiate genotypes of Rhizoctonia solani anastomosis group 3 subgroup PT (AG-3 PT), a fungal pathogen of potato. Polymorphic co-dominant single-locus PCR-RFLP markers were identified after sequencing of clones from a genomic library and digestion with restriction enzymes. Multilocus genotypes were determined by a combination of PCR product and digestion with a specific restriction enzyme for each of seven loci. A sample of 104 isolates from one commercial field in each of five counties in eastern North Carolina was analyzed, and evidence for high levels of gene flow between populations was revealed. When data were clone-corrected and samples pooled into one single North Carolina population, random associations of alleles were found for all loci or pairs of loci, indicating random mating. However, when all genotypes were analyzed, the observed genotypic diversity deviated from panmixia and alleles within and between loci were not randomly associated. These findings support a model of population structure for R. solani AG-3 PT on potato that includes both recombination and clonality.


http://www.mycologia.org/cgi/content/abstract/95/6/1100

Species of Trichoderma and Hypocrea that have green conidia and sterile or fertile elongations of their conidiophores are described or redescribed and their phylogenetic position explored. The described species include T. crassum, T. fasciculatum, T. fertile, T. hamatum, T. longipile, T. oblongisporum, T. pubescens, T. spirale, T. strictipile, T. strigosum, T. stromaticum, T. tomentosum, Hypocrea aureoviridis f. macrospora, H. ceramics. and H. semiworbis. Trichoderma fasciculatum originally was described from cultures from ascospores of an unidentified Hypocrea specimen; it is considered to be a synonym of T. strictipile. The remaining species of Trichoderma considered here have not been linked to teleomorphs, and the Trichoderma anamorphs of H. aureoviridis f. macropora and H. semiworbis have not been named. Five new species of Hypocrea are described, viz. H. cremea, H. cuneispore, H. estonica, H. strictipilosa and H. surrotunda. The phylogenetic relationships of these species were inferred based on partial RPB2 and EF-1(α) DNA sequence data and phenotypic characteristics, including teleomorph, anamorph, colony and growth rates. Trichoderma crassum was found to be a sister species to T. virens, based on
molecular sequences and phenotypic data. Hypocrea surrotunda and H. cremea, H. cuneispora and T. longipile, T. fertile and T. oblongisporum, T. tomentosum and H. atrogelatinosa, and T. hamatum and T. pubescens, respectively, were found to be closely related phylogenetically, based on RPB2 and EF-1{alpha} gene genealogies. Anamorph and teleomorph phenotype, including conidiophore elongations, phialide morphology, conidial morphology, stroma anatomy and ascospore morphology are not useful predictors of relationships. Despite the shared phenotypic characters of these Trichoderma and Hypocrea species, they are distributed between two major clades of Trichoderma/Hypocrea. Redescriptions and a key to species of Hypocrea/Trichoderma with green conidia and conidiophore elongations are presented.


http://www.mycologia.org/cgi/content/abstract/95/2/285

Armillaria root rot is a serious disease, chiefly of woody plants, caused by many species of Armillaria that occur in temperate, tropical and subtropical regions of the world. Very little is known about Armillaria in South America and Southeast Asia, although Armillaria root rot is well known in these areas. In this study, we consider previously unidentified isolates collected from trees with symptoms of Armillaria root rot in Chile, Indonesia and Malaysia. In addition, isolates from basidiocarps resembling A. novae-zelandiae and A. limonea, originating from Chile and Argentina, respectively, were included in this study because their true identity has been uncertain. All isolates in this study were compared, based on their similarity in ITS sequences with previously sequenced Armillaria species, and their phylogenetic relationship with species from the Southern Hemisphere was considered. ITS sequence data for Armillaria also were compared with those available at GenBank. Parsimony and distance analyses were conducted to determine the phylogenetic relationships between the unknown isolates and the species that showed high ITS sequence similarity. In addition, IGS-1 sequence data were obtained for some of the species to validate the trees obtained from the ITS data set. Results of this study showed that the ITS sequences of the isolates obtained from basidiocarps resembling A. novae-zelandiae are most similar to those for this species. ITS sequences for isolates from Indonesia and Malaysia had the highest similarity to A. novae-zelandiae but were phylogenetically separated from this species. Isolates from Chile, for which basidiocarps were not found, were similar in their ITS and IGS-1 sequences to the isolate from Argentina that resembled A. limonea. These isolates, however, had the highest ITS and IGS-1 sequence similarity to authentic isolates of A. luteobubalina and were phylogenetically more closely related to this species than to A. limonea.


http://www.mycologia.org/cgi/content/abstract/94/5/814

Stachybotrys chartarum has received much attention as a possible cause of sick-building syndrome. Because morphological species recognition in fungi can hide diversity, we applied a phylogenetic approach to search for cryptic species. We examined 23 isolates from the San Francisco Bay Area, and another seven from around the US. Using markers we developed for three polymorphic protein coding loci (chitin synthase 1, beta-tubulin 2, and trichodiene synthase 5), we infer that two distinct phylogenetic species exist within the single described morphological species. We have found no correlation between genetic isolation and geographic distance.

http://www.mycologia.org/cgi/content/abstract/94/3/494

Forty isolates of Phomopsis were obtained from twigs and berries of highbush blueberry, Vaccinium corymbosum, and cranberry, Vaccinium macrocarpon, isolated primarily from plants grown in the eastern United States. They were characterized using conidiomatal morphology, conidial dimensions, colony appearance and growth rate, and sequences of ITS rDNA. Based on morphological and molecular similarities, most isolates grouped together with an authentic culture of Phomopsis vaccinii Shear. This taxon is described and illustrated. However, some Phomopsis isolates from Vaccinium differed in colony and conidiomatal morphology from P. vaccinii and, based on ITS sequences, were related to isolates of Phomopsis from diverse hosts. These isolates were excluded from P. vaccinii.


http://www.mycologia.org/cgi/content/abstract/95/4/637

Phialocephala was established for species in the Leptographium complex that produce conidia from phialides at the apices of dark mononematous conidiophores. Some species previously included in Phialocephala were re-allocated to Sporendocladia because they resembled Thielaviopsis in having ring-wall-building conidial development and conidia with two attachment points that emerge in false chains. Despite this significant realignment of the genus, a great deal of morphological heterogeneity remains in Phialocephala. The objective of this study was to consider the heterogeneity among Phialocephala spp. based on comparisons of sequence data derived from the large and small subunits (LSU and SSU) of the rRNA operon of species in Phialocephala. Phialocephala dimorphospora, the type species of the genus, and P. fortinii grouped with genera of the Helotiales in phylogenetic trees generated based on the LSU and SSU datasets. Phialocephala xalapensis and P. fusca clearly are unrelated to Phialocephala sensu stricto and should represent a new genus in the Ophiostomatales. Phialocephala compacta resides with representatives of the Hypocreales, and we believe that it represents a distinct genus. Phialocephala scopiformis and P. repens are not closely related to the other Phialocephala species and group within the Dothideales. The morphological heterogeneity among species of Phialocephala clearly is reflected by phylogenetic analysis of sequence data from two conserved rRNA gene regions. Appropriate genera now need to be found to accommodate these fungi.


http://www.mycologia.org/cgi/content/abstract/96/1/66

The fungal genus Neurospora has a distinguished history as a laboratory model in genetics and biochemistry. The most recent milestone in this history has been the sequencing of the genome of the best known species, N. crassa. The hope and promise of a complete genome sequence is a full understanding of the biology of the organism. Full understanding cannot be achieved, however, in the absence of fundamental knowledge of natural history. We report that species of Neurospora, heretofore thought to occur mainly in moist tropical and subtropical regions, are
common primary colonizers of trees and shrubs killed by forest fires in western North America, in regions that are often cold and dry. Surveys in 36 forest-fire sites from New Mexico to Alaska yielded more than 500 cultures, 95% of which were the rarely collected N. discreta. Initial characterization of genotypes both within a site and on a single tree showed diversity consistent with sexual reproduction of N. discreta. These discoveries fill important gaps in knowledge of the distribution of members of the genus on both large and small spatial scales and provide the framework for future studies in new regions and microhabitats. The overall result is that population biology and genetics now can be combined, placing the genus Neurospora in a unique position to expand its role in experimental biology as a useful model organism for ecology, population genetics and evolution.


http://www.mycologia.org/cgi/content/abstract/95/6/1252

Aspergillus ochraceoroseus produces the yellow-gold conidia and other characteristics of Aspergillus subgenus Circumdati section Circumdati. However, this species produces aflatoxin, a secondary metabolite characteristic of some members of subgenus Circumdati section Flavi and sterigmatocystin, a related secondary metabolite usually associated with subgenus Nidulantes sections Nidulantes and Versicolores, as well as members of several other genera. Our morphological data support the placement of A. ochraceoroseus in subgenus Circumdati. Sequence data from A. ochraceoroseus aflatoxin and sterigmatocystin genes aflR and nor-1/stcE, as well as 5.8S ITS and beta tubulin genes, were compared to those of aspergilli in sections Circumdati, Flavi, Nidulantes and Versicolores. In the sequence comparisons, A. ochraceoroseus was related more closely to the species in subgenus Nidulantes than to species from subgenus Circumdati.


http://www.mycologia.org/cgi/content/abstract/94/4/694

Members of the genus Neotyphodium are asexual, seedborne, protective fungal endophytes of cool season grasses that have likely evolved either directly from sexual Epichloe; species, or by the interspecific hybridization of distinct lineages of Epichloe; and Neotyphodium. We investigated the evolutionary origins of Neotyphodium endophytes from several grasses that are indigenous to the Southern Hemisphere using a multiple-gene phylogenetic approach. Intron regions of the genes encoding {beta}-tubulin (tub2), translation elongation factor 1-{alpha} (tef1) and actin (act1) were amplified by polymerase chain reaction and sequenced. Phylogenetic analyses of these sequences, aligned with homologous sequences from Epichloe; spp., revealed the evolutionary origins of the Southern Hemisphere endophytes, where one lineage of apparently non-hybrid origin, and three lineages of unique interspecific hybrid origin were identified. On the basis of morphology, host range and evolutionary history, we propose three new species of Neotyphodium. Neotyphodium aotearoae was isolated from Echinopogon ovatus populations from New Zealand and Australia, and comprised a unique, apparently non-hybrid lineage within the Epichloe; species phylogeny. In contrast, an interspecific hybrid lineage was identified from two Australian Ec. ovatus populations, whose ancestry apparently involved lineages closely related to extant E. festucae and an E. typhina genotype similar to that of isolates from Poa pratensis. Endophytes infecting South African Melica racemosa and M. decumbens (dronkgras) appeared to be hybrids of E. festucae and N. aotearoae or close relatives. The names N. australiense and N. melicicola are proposed for these two hybrid lineages, respectively. The
origin of N. tembladerae, an established endophyte species from South American Poa and Festuca spp., was also investigated. Neotyphodium tembladerae appeared to be of hybrid origin, involving E. festucae and an E. typhina genotype similar to that of isolates from Poa nemoralis. The results of this study highlight the widespread occurrence of interspecific hybrid Neotyphodium lineages on a global scale, and the extent of endophyte gene-flow between the Northern and Southern Hemispheres.


http://www.mycologia.org/cgi/content/abstract/96/5/990

The fungal genera Endothia and Cryphonectria include some of the most important pathogens of forest trees. Despite available new technology, no comprehensive comparative study based on DNA sequence data and morphology has been done on the available isolates representing these two genera. The main objectives of this study were to assess the phylogenetic relationships among species of Cryphonectria and Endothia, for which cultures are available, and to establish a taxonomic framework based on DNA sequence and morphological data, which will aid future studies and identification of species in these and related genera. Comparisons were based on sequence variation found in the ITS region of the ribosomal RNA operon and two regions of the \{beta\}-tu-bulin gene. In addition, the morphology of these species was examined. The phylogenetic data indicated that Endothia and Cryphonectria reside in two distinct phylogenetic clades. Cryphonectria parasitica, C. macrospora, C. nitschkei, C. eucalypti and C. radicalis represented the Cryphonectria clade. Endothia gyrosa and E. singularis were included in the Endothia clade. An isolate representing E. viridistromata grouped outside the Endothia clade and separately from other groups. Other clades outside the one encompassing Cryphonectria were those represented by the C. cubensis isolates and fungi isolated from Elaeocarpus dentatus originating from New Zealand. These clades could be distinguished from Endothia and Cryphonectria, based on anamorph morphology, stromatal structure and ascospore septation. Cryphonectria and Endothia, therefore, appear to be paraphyletic and taxonomic relationships for these fungi need to be revised.


http://www.mycologia.org/cgi/content/abstract/95/5/921

Of the eight species of Hypomyces that occur on basidiomata of Stereum species, only H. sympodiophorus grows exclusively on members of this genus. Morphologically similar fungi were found on species of Xylobolus, a genus closely related to Stereum. These are described as two new species of Hypomyces: H. thailandicus, collected on Xylobolus cf. illudens in Thailand; and H. xyloboli, on X. frustulatus and X. subpileatus in the eastern United States. These three species are unusual in Hypomyces because of their almost indistinguishable anamorphs. In parsimony analysis of LSU nuclear rDNA sequences, the three species growing only on Stereaceae do not form a monophyletic group but their constrained monophyly is not rejected either. A morphologically similar anamorphic species, Sibirina gamsii, included in the study, is transferred to the genus Cladobotryum.


http://www.mycologia.org/cgi/content/abstract/95/5/809

Although species of Neurospora are among the most studied model organisms in genetics and biochemistry, basic questions remain with respect to their ecology and population biology. In this study, we sought to clarify relationships among individuals over a small spatial scale, toward assessing both local variation and mode of colonization. Isolates of Neurospora were collected after fires in the Florida Everglades (May 1999), where abundant colonies appeared on diverse plants, including grasses and woody shrubs. Colonies were sampled in a linear fashion from two adjacent scorched sugarcane stems at one site and from a burned woody shrub at a distant second site. Species and mating types were assigned based on crossing behavior. Variation at two loci, het-c and frq, was determined by direct sequencing of PCR products. The results demonstrated substantial within- and among-species variation on a small scale, with up to three species and six different haplotypes occurring on a single stem. In total, four species and more than 10 genetically distinct individuals (haplotypes) were present across the three stems, often with multiple individuals occupying the same position. A permutation analysis revealed that individuals were not distributed randomly and that adjacent nodes on cane stems were more likely than chance to be colonized by the same haplotype. This suggests that visible eruptions of conidia on burned plants reflect substantial vegetative mycelial spread through subsurface tissues after primary colonization. Results also revealed that adjacent isolates from a single plant can possess different functional alleles at het-c, an observation meaningful in the context of the proposed role of het-c in self recognition.


http://www.mycologia.org/cgi/content/abstract/94/1/49

Alternaria radicina, A. carotiincultae, and A. petroselini are closely related pathogens of umbelliferous crops. Relationships among these fungi were determined based on growth rate, spore morphology, cultural characteristics, toxin production, and host range. Random amplified polymorphic DNA (RAPD) analysis of these species, other species of Alternaria, and closely related fungi was also performed. A. petroselini was readily differentiated from A. radicina and A. carotiincultae on the basis of spore morphology, production of microsclerotia, host range, and RAPD analysis. Alternaria radicina and A. carotiincultae were considerably more similar to each other than to A. petroselini, but could be differentiated on the basis of growth rate, spore morphology, colony morphology, and, to a limited extent, RAPD analysis. When grown on media having a high nutritional content, A. radicina produced a diffusible yellow pigment and crystals of the fungal metabolite radicinin. In contrast, A. carotiincultae produced little or no radicinin. However, when A. carotiincultae was grown on the same medium amended with radicinin, growth rate and colony and conidial morphology were more similar to those of A. radicina. These results suggest that the morphological differences between A. radicina and A. carotiincultae are due, at least in part, to radicinin production, and that these fungi are conspecific. Therefore, we propose that A. carotiincultae be considered a synonym of A. radicina.


http://www.mycologia.org/cgi/content/abstract/95/5/827
The phylogenetic relationships of the lichen genus Placopsis and related genera in the Agyriales were analyzed using molecular data. We obtained a total of 66 new sequences from the nuclear ITS, LSU and the mitochondrial SSU rDNA. Phylogenetic analyses were conducted in a Bayesian and a maximum-parsimony framework. Our analyses show that Placopsis is paraphyletic with members of Orceolina nesting within the genus. A morphological character supporting the Placopsis-Orceolina clade is the non-amyloid ascus. The section Aspiciliopsis as defined by sunken fruiting bodies is not supported, but the type species of Aspiciliopsis is more closely related to Orceolina. This clade shares apothecia with reduced amphithecia as apomorphic character. We suggest resurrecting the generic name Aspiciliopsis. Trapelia is the sister genus to Placopsis and Aspiciliopsis/Orceolina.


http://www.mycologia.org/cgi/content/abstract/96/6/1330

Current taxonomy places all rust fungi that occur on willow (Salix spp.) in North America in one species complex, Melampsora epitea Thum. Characteristics of M. epitea isolates from the Canadian arctic were compared to M. epitea isolates from temperate regions of North America. Sequences from internal transcribed spacer (ITS) regions of rDNA were obtained from urediniospores from rust-infected Salix leaves collected in the Canadian arctic and in Minnesota and compared. Phylogenetic analysis of nuclear ribosomal ITS regions indicated that arctic M. epitea samples were divergent from temperate M. epitea isolates, perhaps in part because all rusts examined diverged according to host species. Four urediniospore characteristics were examined: area, circularity (shape factor), major axis length and spine density. Statistically significant (P < 0.05) differences were observed for spine density among all host species except S. nigra and S. bebbiana. However major axis length differed between these species. These results represent the first evidence that arctic and temperate Melampsora species on Salix hosts in North America have evolved distinct molecular and morphological characters.


http://www.mycologia.org/cgi/content/abstract/96/4/781

Several species of Botryosphaeria are known to occur on grapevines, causing a wide range of disorders including bud mortality, dieback, brown wood streaking and bunch rot. In this study the 11 Botryosphaeria spp. associated with grapevines growing in various parts of the world, but primarily in South Africa, are distinguished based on morphology, DNA sequences (ITS-1, 5.8S, ITS-2 and EF1-{alpha}) and pathological data. Botryosphaeria australis, B. lutea, B. obtusa, B. parva, B. rhodina and a Diplodia sp. are confirmed from grapevines in South Africa, while Diplodia porosum, Fusicoccum viticlavatum and F. vitifusiforme are described as new. Although isolates of B. dothidea and B. stevensii are confirmed from grapevines in Portugal, neither of these species occurred in South Africa, nor were any isolates of B. ribis confirmed from grapevines. All grapevine isolates from Portugal, formerly presumed to be B. ribis, are identified as B. parva based on their EF1-{alpha} sequence data. From artificial inoculations on grapevine shoots, we conclude that B. australis, B. parva, B. ribis and B. stevensii are more virulent than the other species studied. The Diplodia sp. collected from grapevine canes is morphologically similar but phylogenetically distinct from D. sarmentorum. Diplodia sarmentorum is confirmed as anamorph of Otthia spiraeae, the type species of the genus Otthia (Botryosphaeriaceae). A culture identified as O. spiraeae clustered within Botryosphaeria and thus is regarded as probable
These findings confirm earlier suggestions that the generic concept of Botryosphaeria should be expanded to include genera with septate ascospores and Diplodia anamorphs.

N. Engl. J. Med. (4)


http://content.nejm.org/cgi/content/abstract/350/12/1179

Background Recent outbreaks of avian influenza A (H5N1) in poultry throughout Asia have had major economic and health repercussions. Human infections with this virus were identified in Vietnam in January 2004. Methods We report the clinical features and preliminary epidemiologic findings among 10 patients with confirmed cases of avian influenza A (H5N1) who presented to hospitals in Ho Chi Minh City and Hanoi, Vietnam, in December 2003 and January 2004. Results In all 10 cases, the diagnosis of influenza A (H5N1) was confirmed by means of viral culture or reverse transcriptase-polymerase chain reaction with primers specific for H5 and N1. None of the 10 patients (mean age, 13.7 years) had preexisting medical conditions. Nine of them had a clear history of direct contact with poultry (median time before onset of illness, three days). All patients presented with fever (temperature, 38.5 to 40.0 degrees C), respiratory symptoms, and clinically significant lymphopenia (median lymphocyte count, 700 per cubic millimeter). The median platelet count was 75,500 per cubic millimeter. Seven patients had diarrhea. In all patients, there were marked abnormalities on chest radiography. There was no definitive evidence of human-to-human transmission. Eight patients died, one patient has recovered, and one is recovering. Conclusions Influenza A (H5N1) infection, characterized by fever, respiratory symptoms, and lymphopenia, carries a high risk of death. Although in all 10 cases the infection appears to have been acquired directly from infected poultry, the potential exists for genetic reassortment with human influenza viruses and the evolution of human-to-human transmission. Containment of influenza A (H5N1) in poultry throughout Asia is therefore urgently required.


http://content.nejm.org/cgi/content/abstract/347/2/95

Background Several genetic defects are associated with permanent congenital hypothyroidism. Immunologic, environmental, and iatrogenic (but not genetic) factors are known to induce transient congenital hypothyroidism, which spontaneously resolves within the first months of life. We hypothesized that molecular defects in the thyroid oxidase system, which is composed of at least two proteins, might be involved in the pathogenesis of permanent or transient congenital hypothyroidism in babies with defects in iodide organification, for which the oxidase system is required. Methods Nine patients were recruited who had idiopathic congenital hypothyroidism (one with permanent and eight with transient hypothyroidism) and an iodide-organification defect and who had been identified by the screening program for congenital hypothyroidism. The DNA of the patients and their relatives was analyzed for mutations in the genes for thyroid oxidase 1 (THOX1) and 2 (THOX2). Results The one patient with permanent and severe thyroid hormone deficiency and a complete iodide-organification defect had a homozygous nonsense mutation in
the THOX2 gene that eliminates all functional domains of the protein. Three of the eight patients with mild transient congenital hypothyroidism and a partial iodide-organification defect had heterozygous mutations in the THOX2 gene that prematurely truncate the protein, thus abolishing its functional domains. Conclusions Biallelic inactivating mutations in the THOX2 gene result in complete disruption of thyroid-hormone synthesis and are associated with severe and permanent congenital hypothyroidism. Monoallelic mutations are associated with milder, transient hypothyroidism caused by insufficient thyroidal production of hydrogen peroxide, which prevents the synthesis of sufficient quantities of thyroid hormones to meet the large requirement for thyroid hormones at the beginning of life.


http://content.nejm.org/cgi/content/abstract/347/15/1151

Background The innate immune system of human skin contains antimicrobial peptides known as cathelicidins (LL-37) and {beta}-defensins. In normal skin these peptides are negligible, but they accumulate in skin affected by inflammatory diseases such as psoriasis. We compared the levels of expression of LL-37 and human {beta}-defensin 2 (HBD-2) in inflamed skin from patients with atopic dermatitis and from those with psoriasis. Methods The expression of LL-37 and HBD-2 protein in skin-biopsy specimens from patients with psoriasis, patients with atopic dermatitis, and normal subjects was determined by immunohistochemical analysis. The amount of antimicrobial peptides in extracts of skin samples was also analyzed by immunodot blot analysis (for LL-37) and Western blot analysis (for HBD-2). Quantitative, real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays were used to confirm the relative expression of HBD-2 and LL-37 messenger RNA (mRNA) in the skin-biopsy specimens. These peptides were also tested for antimicrobial activity against Staphylococcus aureus with the use of a colony-forming assay. Results Immunohistochemical analysis confirmed the presence of abundant LL-37 and HBD-2 in the superficial epidermis of all patients with psoriasis. In comparison, immunostaining for these peptides was significantly decreased in acute and chronic lesions from patients with atopic dermatitis (P=0.006 and P=0.03, respectively). These results were confirmed by immunodot blot and Western blot analyses. Real-time RT-PCR showed significantly lower expression of HBD-2 mRNA and LL-37 mRNA in atopic lesions than in psoriatic lesions (P=0.009 and P=0.02, respectively). The combination of LL-37 and HBD-2 showed synergistic antimicrobial activity by effectively killing S. aureus. Conclusions A deficiency in the expression of antimicrobial peptides may account for the susceptibility of patients with atopic dermatitis to skin infection with S. aureus.


http://content.nejm.org/cgi/content/abstract/346/2/99

Background Progressive osseous heteroplasia (POH), an autosomal dominant disorder, is characterized by extensive dermal ossification during childhood, followed by disabling and widespread heterotopic ossification of skeletal muscle and deep connective tissue. Occasional reports of mild heterotopic ossification in Albright's hereditary osteodystrophy (AHO) and a recent report of two patients with AHO who had atypically extensive heterotopic ossification suggested a common genetic basis for the two disorders. AHO is caused by heterozygous inactivating mutations in the GNAS1 gene that result in decreased expression or function of the alpha subunit of the stimulatory G protein (Gs(alpha)) of adenyl cyclase. Methods We tested the hypothesis that GNAS1 mutations cause POH, using the polymerase chain reaction to amplify GNAS1 exons...
and exon-intron boundaries in 18 patients with sporadic or familial POH. Results Heterozygous inactivating GNAS1 mutations were identified in 13 of the 18 probands with POH. The defective allele in POH is inherited exclusively from fathers, a result consistent with a model of imprinting for GNAS1. Direct evidence that the same mutation can cause either POH or AHO was observed within a single family, in which the phenotype correlated with the parental origin of the mutant allele. Conclusions Paternally inherited inactivating GNAS1 mutations cause POH. This finding extends the range of phenotypes derived from haploinsufficiency of GNAS1, provides evidence that imprinting is a regulatory mechanism for GNAS1 expression, and suggests that Gs(alpha) is a critical negative regulator of osteogenic commitment in nonosseous connective tissues.

Nephrol. Dial. Transplant. (8)


http://ndt.oupjournals.org/cgi/content/abstract/18/4/710

Background. In the glomerular mesangium, immunologic and/or infectious activation of the inflammatory, NF-(kappa)B-mediated signal pathway can induce a progression of already existing mesangial lesions in non-immunologic and immunologic glomerular disease. This progression is preceded by upregulated mesangial gene expression of which the vascular cell adhesion molecule-1, VCAM-1 (vascular cell adhesion molecule-1), is a well-established marker. Its evaluation on minimal tissue such as routinely paraffinized needle core biopsies is not established and needs the development of a novel evaluation method more meaningful than common immunohistology. Methods. By laser-microdissection, 10 glomeruli/case were isolated from 5 (micro)m thick tissue slices in a total of 15 cases of mesangial proliferation with different renal diseases (IgA nephropathy, lupus nephritis and mesangial proliferative lesions of unknown aetiology) vs transplant biopsies as negative and TNF (alpha)-treated cultured human mesangial cells as positive controls. After reverse transcription of isolated RNA, cDNA aliquots were quantified for VCAM-1 expression by real-time PCR using the threshold cycle (Ct) method, normalized for the housekeeping gene (beta)-actin, and compared with qualitative RT-PCR results. Results. Unsuspected VCAM-1 transcript steady-state levels could be detected by real-time PCR in agreement with qualitative PCR, while morphologic and immunhistologic analyses were unrevealing. As yields of RNA extraction in femtogram quantities cannot be measured spectrophotometrically, a Ct-ratio was formed between (beta)-actin and VCAM-1 per case showing high VCAM-1 expression in lupus nephritis (1.39), and moderate expression in IgA nephropathy (1.08-1.23) vs TNF (alpha)-treated mesangial cells (0.97-1.23) and negative control cases (0.66-0.68). Conclusions. This is the first reported gene expression analysis method for routinely paraffinized human renal biopsies, demonstrating the power of combined laser-microdissection and PCR quantification as novel methods for the evaluation of minimal tissue beyond purely descriptive morphologic analysis.

Background. Tubulointerstitial fibrosis is an important component of diabetic nephropathy, which is characterized by increased expression of interstitial extracellular matrix components and aberrant expression of the basement membrane component collagen type IV. The present study examined the effect of high ambient glucose and transforming growth factor-β1 (TGF-β1) on collagen secretion by human renal fibroblasts and proximal tubular epithelial cells (PTECs). Methods. Human renal fibroblasts (TK173) and PTECs (HK2) were used to examine the effects of high glucose (25 mM D-glucose) and TGF-β1 (1 ng/ml) on collagen type I, III and IV secretion compared with control medium (5.5 mM glucose). Matrix components were measured by enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR). Results. Renal fibroblasts are the main producers of the interstitial components collagen type I and type III, while collagen type IV was secreted predominantly by PTECs. However, renal fibroblasts were also able to secrete collagen type IV. Secretion of collagen type IV by fibroblasts was increased upon stimulation with TGF-β1, reaching levels comparable with those secreted by TGF-β1-induced PTECs. Moreover, high glucose stimulated increased collagen type IV secretion. Importantly, this could not be attenuated by neutralizing pan-specific anti-TGF-β antibodies. Conclusions. These data show that renal fibroblasts secrete collagen type IV, which can be increased by high glucose independent of endogenous TGF-β1. This suggests that as well as the increased expression of interstitial components, renal fibroblasts can contribute to the increased expression of the basement membrane component collagen type IV in tubulointerstitial fibrosis observed during diabetic nephropathy.


Background. The pathogenetic mechanisms responsible for progressive renal impairment of diabetic nephropathy are still poorly understood, despite its growing incidence. Increasing evidence suggests that growth factors may contribute to the initiation and progressive fibrosis of diabetic nephropathy. In this study, the gene expression and protein distribution of platelet-derived growth factor-A and -B (PDGF-A and PDGF-B) in human diabetic nephropathy were examined. Methods. PDGF-A and PDGF-B mRNA levels in surplus renal biopsy tissue from seven patients with overt diabetic nephropathy and six nephrectomy samples were examined using quantitative reverse transcription-polymerase chain reaction (RT-PCR). In addition, each sample was also examined immunohistochemically to quantify and localize peptide expression of each PDGF isoform. Results. Gene expression of PDGF-A and PDGF-B mRNA were increased 22- and 6-fold, respectively, in biopsies from patients with diabetic nephropathy compared with control tissue. Immunostaining also demonstrated increased peptide expression of both PDGF-A and PDGF-B in diabetic nephropathy, with each isoform showing a specific pattern of tissue distribution. Conclusions. The findings of increased gene and protein expression of PDGF in renal biopsies from patients with diabetic nephropathy imply a potential role for this prosclerotic growth factor in the development of the progressive fibrosis that characterizes human diabetic kidney disease.

Background. It has been suggested that changes in immune response to infectious agents in patients on haemodialysis might be due to impaired monocyte function; uraemic and haemodialysed patients overproduce proinflammatory cytokines, such as interleukin-1 beta (IL-1{beta}), tumor necrosis factor-alpha (TNF-{alpha}) and interleukin-6 (IL-6). Methods. We quantitated the cytokines released into the plasma and into the supernatants of 24-h cultured purified monocytes, under basal conditions and after stimulation by lipopolysaccharide from Escherichia coli, in 15 healthy subjects (CON), 20 uraemic patients who had not yet started dialysis (CRF) and 60 haemodialysed patients (HD), who were divided into three groups of 20 patients corresponding to short-, medium- and long-term dialysis. Results. Monocytes from HD patients spontaneously secreted significantly higher levels of cytokines than those from controls and uraemic patients who had not yet started dialysis. After stimulation with lipopolysaccharide (LPS), cytokine levels in culture supernatants of cells from HD patients were significantly lower than those from controls and uraemic patients. Moreover, levels of cytokines in monocyte supernatants and plasma from short-, medium- and long-term haemodialysed patients decreased progressively with dialytic age. Monocytes from haemodialysed patients tended to be constitutively active, but their ability to secrete proinflammatory cytokines was inversely correlated with dialytic age. Conclusions. These results indicate that prolonged treatment with dialysis can be considered a form of chronic stress that causes the progressive activation of monocytes, which ultimately leads to monocyte exhaustion and dysfunction.


http://ndt.oupjournals.org/cgi/content/abstract/19/4/797

Background. Although hypoalbuminaemia is a significant predictor of mortality in haemodialysis (HD) patients, the pathophysiological mechanisms involved remain to be determined. Albumin is a negative acute-phase reactant and many proinflammatory substances are elevated in HD patients. We investigated factors that may affect liver albumin synthesis. Methods. Hepatocytes were isolated from rat livers and were cultured with interleukin (IL)-4, IL-6, IL-12, tumor necrosis factor (TNF)-{alpha}, procalcitonin (PCT), a sensitive marker of infection, and indoxyl sulphate (IS), a uraemic toxin. Albumin levels in the supernatant were measured by enzyme-linked immunosorbent assay. Albumin mRNA expression was determined by reverse transcriptase polymerase chain reaction. Results. IL-6 and TNF-{alpha} significantly decreased albumin levels in a dose-dependent manner (P<0.01 and P<0.05, respectively). In contrast, IL-4 and IL-12 did not modulate albumin production. PCT and IS significantly and dose-dependently increased albumin levels (both P<0.01). PCT increased albumin mRNA expression in the hepatocytes (P = 0.05) and dose-dependently abrogated IL-6-induced suppression of albumin synthesis (P<0.01). IS also blocked the IL-6-induced decrease in net albumin secretion (P<0.01). Conclusion. Our findings indicate that PCT and IS protect against suppression of hepatic albumin synthesis caused by proinflammatory cytokines, suggesting their potential role in preventing hypoalbuminaemia in HD patients.


http://ndt.oupjournals.org/cgi/content/abstract/19/10/2499

Background. The serum- and glucocorticoid-regulated kinase (SGK1) gene is an important mediator of aldosterone action, regulating the expression of the renal epithelial Na+ channel. In renal failure, blood pressure (BP) is markedly salt-dependent and increases with decreasing renal
function. Mutations of the SGK1 gene affecting phosphorylation could be responsible for salt-mediated increases in BP and hypertension-related progression to end-stage renal disease (ESRD). Methods. The SGK1 gene was analysed for mutations in the exons 4, 5, 8 and 10-12, because of potential phosphorylation sites, in 591 subjects, including 311 ESRD patients (either dialysis or transplanted). In addition, an intron 6 single-nucleotide polymorphism (SNP) described previously was also investigated in this study. Genotyping was performed either by using a strategy based on single strand conformation polymorphism analysis of polymerase chain reaction (PCR) products and subsequent direct sequencing of identified gel shift variants or by using high throughput 5’ nuclease allelic discrimination assay. Results. Two SNPs in coding regions of SGK1 potentially influencing the phosphorylation of Sgk1 were identified. Both SNPs were synonymous. The prevalence of the first variant, a previously reported SNP at codon 240 in exon 8, did not differ between ESRD patients (16.3%) and controls (15.7%). There was no association between the SNP in exon 8 and either BP within the control population or progression of renal disease in the ESRD population. The second SNP at codon 398 in exon 12 was identified in one patient only. Intron 6 and exon 8 SNPs were in strong linkage disequilibrium, but did not show any association with either BP or renal diseases. Conclusions. Based on statistical analysis homozygosity for nonconservative mutations in the coding region of the SGK1 gene is estimated at <1/300 000 when a white Caucasian population is considered, arguing against an important role of mutations of this coding region in hypertension and hypertension-associated progression of renal disease.


http://ndt.oupjournals.org/cgi/content/abstract/20/5/870

Background. Our earlier studies have demonstrated upregulation of monocyte chemoattractant protein-1 (MCP-1) in NRK52E rat renal epithelial cells by exposure to oxalate (Ox) ions and crystals of calcium oxalate monohydrate (COM) or the brushite (Br) form of calcium phosphate. The upregulation was mediated by reactive oxygen species (ROS). This study was performed to investigate whether NADPH oxidase is involved in ROS production. Methods. Confluent cultures of NRK52E cells were exposed to Ox ions or COM and Br crystals. They were exposed for 1, 3, 6, 12, 24 and 48 h for isolation of MCP-1 mRNA and 24 h for enzyme-linked immunosorbent assay (ELISA) to determine the secretion of protein into the culture medium. We also investigated the effect of free radical scavenger, catalase, and the NADPH oxidase inhibitor diphenyleneiodium (DPI) chloride, on the Ox- and crystal-induced expression of MCP-1 mRNA and protein. The transcription of MCP-1 mRNA in the cells was determined using real-time polymerase chain reaction. Hydrogen peroxide and 8-isoprostane were measured to investigate the involvement of ROS. Results. Exposure of NRK52E cells to Ox ions as well as the crystals resulted in increased expression of MCP-1 mRNA and production of the chemoattractant. Treatment with catalase reduced the Ox- and crystal-induced expression of both MCP-1 mRNA and protein. DPI reduced the crystal-induced gene expression and protein production but not Ox-induced gene expression and protein production. Conclusions. Exposure to Ox ions, and COM and Br crystals stimulates a ROS-mediated increase in MCP-1 mRNA expression and protein production. Reduction in ROS production, lipid peroxidation, low-density lipoprotein release, and inducible MCP-1 gene and protein in the presence of DPI indicates an involvement of NADPH oxidase in the production of ROS.

Background. During the development of non-infectious kidney stones, crystals form and deposit in the kidneys and become surrounded by monocytes/macrophages (M/M). We have proposed that in response to crystal exposure renal epithelial cells produce chemokines, which attract the M/M to the sites of crystal deposition. We investigated the expression of monocyte chemoattractant protein-1 (MCP-1) mRNA and protein by NRK52E rat renal tubular epithelial cells exposed to calcium oxalate (CaOx), brushite (Br, a calcium phosphate) and uric acid (UA) crystals. Methods. Confluent cultures of NRK52E cells were exposed to CaOx, Br or UA at a concentration of 250 (micro)g/ml (66.7 (micro)g/cm2). They were exposed for 1, 3, 6, 12, 24 and 48 h for isolation of mRNA and 24 h for ELISA to determine the secretion of protein into the culture medium. Since cells are known to produce free radicals on exposure to CaOx crystals we also investigated the effect of free radical scavenger catalase on the crystal induced expression of MCP-1 mRNA and protein. Results. Exposure of NRK52E cells to the crystals resulted in increased expression of MCP-1 mRNA and production of the chemoattractant. CaOx crystals were most provocative while UA the least. Treatment with catalase had a negative effect on the increased expression of both MCP-1 mRNA and protein, which indicates the involvement of free radicals in up-regulation of MCP-1 production. Conclusion. Exposure to both CaOx and calcium phosphate crystals stimulates increased production of MCP-1. Free radicals appear to be involved in this up-regulation. Results indicate that MCP-1, which is often associated with localized inflammation, may be one of the chemokine mediators associated with the deposition of various urinary crystals in the kidneys during kidney stone formation. Because of the small number of experiments performed here, results must be confirmed by more extensive studies with larger sample size.

**Neurobiology of Aging** (5)


http://www.sciencedirect.com/science/article/B6T09-49YDBDW-4/2/b8369a1c0ea3d80bc0de19fe6d76404

Increasing evidence suggests that cholesterol plays a central role in the pathophysiology of Alzheimer's disease (AD). Caveolin is a cholesterol-binding membrane protein involved in cellular cholesterol transport. We investigated the changes in the protein amount of hippocampal caveolin of autopsy-confirmed AD and aged-matched control subjects. Our results demonstrate that caveolin protein levels in the hippocampus and caveolin mRNA in the frontal cortex are up-regulated in AD by approximately two-fold, compared to control brains. These results suggest a relationship between caveolin-1 expression levels and a dysregulation of cholesterol homeostasis at the plasma membrane of brain cells. In support of this hypothesis, a significant increase in caveolin protein levels has also been observed in hippocampal tissue from ApoE-deficient (knockout) and aged wild-type mice; two situations associated with modifications of transbilayer distribution of cholesterol in brain synaptic plasma membranes. These results indicate that caveolin over-expression is linked to alterations of cholesterol distribution in the plasma membrane of brain cells and are consistent with the notion of a deterioration of cholesterol homeostasis in AD.
The amplitudes of many circadian rhythms, at the behavioral, physiological, cellular, and biochemical levels, decrease with advanced age. Previous studies suggest that the amplitude of the central circadian pacemaker is decreased in old animals. Recently, it has been reported that expression of several circadian clock genes, including Clock, is lower in the master circadian pacemaker of old rodents. To test the hypothesis that decreased activity of a circadian clock gene renders animals more susceptible to the effects of aging, we analyzed the circadian rhythm of locomotor activity in young and old wild-type and heterozygous Clock mutant mice. We found that the effects of age and the Clock mutation were additive. These results indicate that age-related changes in circadian rhythmicity occur equally in wild-type and heterozygous Clock mutants, suggesting that the Clock mutation does not render mice more susceptible to the effects of age on the circadian pacemaker.

Despite biological support for a role of angiotensin converting enzyme (ACE) in Alzheimer's disease (AD), studies assessing the ACE I/D polymorphism in AD are conflicting. We re-evaluated this association in the Rotterdam Study, a population-based cohort study. The mechanism of association was further explored by adjusting for vascular factors, and by analysing atrophy, white matter lesions and infarcts on MRI in non-demented individuals. Genotypes were available for 6488 participants. During average follow-up of 6 years 250 subjects developed AD. MRI data were available for 494 non-demented participants. Homozygosity for the I-allele conferred a slightly increased risk of AD compared to carrying a D-allele (RR 1.12 (95% CI 0.99-1.25)). This increase was only significant in women, and independent of vascular factors (RR 1.39 (95% CI 1.14-1.69)). Non-demented women with the II genotype had smaller hippocampal and amygdalar volumes. Vascular pathology was not significantly associated with ACE. This suggests a modest but significant increase in risk of AD and early AD pathology in women homozygous for the ACE I-allele independent of vascular factors.

Messenger RNA (mRNA) molecules encoding proteins related to the presynaptic cholinergic and neurotrophin systems were quantitated in the hippocampus and basal forebrain of Long-Evans rats with spatial learning ability assessed in the Morris water maze. The reverse transcriptase-polymerase chain reaction showed that the mRNAs for the low-affinity neurotrophin receptor (p75-NTR) and the growth-associated protein GAP-43 were decreased in level in the basal forebrain of aged-impaired rats. In the hippocampus of these aged-impaired rats, the mRNA for VGF, another neurotrophin-inducible gene, also was decreased. In situ hybridization histochemistry revealed that mRNAs for nerve growth factor (NGF) and brain-derived
neurotrophic factor increased in level in the aged rat hippocampus; when age effects were removed, NGF mRNA level remained significantly correlated with maze performance. Enzyme-linked immunosorbent assay indicated that NGF protein was expressed at normal levels in the aged rat hippocampus. These mRNA and protein alterations may signify that a defect in neurotrophin signaling exists in the brains of aged Long-Evans rats, underlying reduced plasticity responses in the basal forebrain cholinergic system.


Plaques found in the brains of patients suffering from Alzheimer's disease (AD) mainly consist of [beta]-amyloid (A[beta]), which is produced by sequential cleaving of amyloid precursor protein (APP) by two proteolytic enzymes, [beta]- and [gamma]-secretases. Any change in the fine balance between these enzymes and their substrate may contribute to the etio-pathogenesis of AD. Indeed, the protein level and enzymatic activity of [beta]-secretase (BACE), but not its mRNA level, were found elevated in brain areas of AD patients who suffer a high load of A[beta] plaque formation. Similarly, increased BACE activity but no mRNA change was observed in a transgenic mouse model of AD, tg2576, in which over expression of the Swedish mutated human APP leads to A[beta] plaque formation and learning deficits. Based on the recent demonstration of four BACE splice variants with different enzymatic activity, the discrepancy between BACE activity and mRNA expression may be explained by the altered BACE alternative splicing. To test this hypothesis, we studied the expression of all BACE splice variants in different brain areas of tg2576 mice at age of 4 months and 1 year old. We found developmental and regional differences between wild-type and tg2576 mice. Our results indicate that over expression of APP in tg2576 mice leads to the altered alternative splicing of BACE and the increase of its enzymatically more active splice variant (I-501).

Neurobiology of Disease (5)


CST3 is the coding gene for cystatin C (CysC). CST3 B/B homozygosity is associated with an increased risk of developing Alzheimer disease. We performed CysC analysis on human primary skin fibroblasts obtained from donors carrying A/A, A/B, and B/B CST3. Pulse-chase experiments demonstrated that the release of the B variant of CysC has a different temporal pattern compared to that of the A one. Fibroblasts B/B homozygous displayed a reduced secretion of CysC due to a less efficient cleavage of the signal peptide, as suggested by high-resolution Western blot analysis and by in vitro assay. In the brain, the reduced level of CysC may represent the molecular factor responsible for the increased risk of Alzheimer disease.

Experimental autoimmune neuritis (EAN) is an inflammatory autoimmune demyelinating disease of peripheral nervous system (PNS) and represents an animal model of Guillain-Barre syndrome (GBS) in man. The inflammatory cell infiltrating into the PNS is a prerequisite for developing EAN. To explore the role of CC chemokine receptor 5 (CCR5) in the inflammatory process of EAN, we induced EAN in CCR5-deficient (CCR5-/-) mice with P0 protein peptide 180-199. We found that CCR5-/- mice showed a similar EAN clinical course and severity as well as profile of infiltrating macrophages and T cells in cauda equina (CE) of EAN and the same levels of spleen mononuclear cell (MNC) response to antigen and mitogen when compared with CCR5+/+ control mice. However, increased IP-10 and MIP-1[beta] production in sciatic nerves were seen in CCR5-/ mice. These results suggest that CCR5 deficiency does not prevent P0 peptide 180-199-immunized mice from EAN. Increased MIP-1[beta] and IP-10 in sciatic nerves may compensate the CCR5 deficiency and contribute to inflammatory cells infiltrating to the PNS.


The mutilated-foot rat (mf rat) is an autosomal recessive mutant with characteristic digit deformities in adult animals, and this phenotype mimics many aspects of human sensory neuropathy. The genetics of mf rats was recently elucidated. To understand whether the genotype is responsible for cutaneous denervation before clinically overt mutilation in adult mf rats, we investigated skin innervation in postnatal day 7 (P7) mf rats and compared the patterns with P7 wild-type rats. The mf rat carries a G->A mutation in the gene encoding the delta subunit of the cytosolic chaperonin-containing t-complex peptide-1 (Cct4). In the footpad skin of P7 mf rats, there was a >90% loss of epidermal nerves (0.7-7.9% of P7 wild-type rats) as indicated by neuronal markers including protein gene product 9.5 (PGP 9.5), growth-associated protein 43 (GAP43), calcitonin gene-related peptide (CGRP), and substance P (SP). The epidermis of hairy skin in hind feet was completely denervated in mf rats as well. Compared with an approximately 80% reduction in the size of dermal nerve fascicles and a parallel loss of nerve fibers, the nearly complete absence of epidermal innervation suggests further sensory nerve degeneration at the level of nerve terminals in the epidermis. In contrast, the loss of epidermal nerves in the abdominal skin of mf rats was less extensive than that in the footpad skin of mf rats; CGRP (+) and SP (+) fibers were moderately reduced (28.3-56.4% of levels of wild-type rats) with normal amounts of PGP 9.5 (+) and GAP43 (+) nerves. Sympathetic innervation as assessed by tyrosine hydroxylase immunoreactivity was absent from the footpad and abdominal skin of mf rats. In conclusion, there is regional skin denervation with diffuse sympathetic denervation in P7 mf rats. These results suggest that the mutation in Cct4 underlies cutaneous nerve degeneration in mf rats.

A Swiss frontotemporal dementia (FTD) kindred with extrapyramidal-like features and without motor neuron disease shows a brain pathology with ubiquitin-positive but tau-negative inclusions. Tau and neurofilament modifications are now studied here in three recently deceased family members. No major and specific decrease of tau was observed as described by others in, e.g., sporadic cases of FTD with absence of tau-positive inclusions. However, a slight decrease of tau, neurofilament, and synaptic proteins, resulting from frontal atrophy was detected. In parallel, polymorphic markers on chromosome 17q21-22, the centromeric region of chromosome 3 and chromosome 9, were tested. Haplotype analysis showed several recombination events for chromosomes 3 and 17, but patients shared a haplotype on chromosome 9q21-22. However as one of the patients exhibited Alzheimer and vascular dementia pathology with uncertain concomitant FTD, this locus is questionable. Altogether, these data indicate principally that the Swiss kindred is unlinked to locus 17q21-22, and that tau is not at the origin of FTD in this family.


Astroglial glutamate transporters, GLT-1 and GLAST, play an essential role in removing released glutamate from the extracellular space and are essential for maintaining a low concentration of extracellular glutamate in the brain. It was hypothesized that impaired function of glial glutamate transporters induced by transient global ischemia may lead to an elevated level of extracellular glutamate and subsequent excitotoxic neuronal death. To test this hypothesis, in the present study, we performed whole-cell patch-clamp recording of hippocampal CA1 astrocytes in control or postischemic slices, and measured glutamate transporter activity by recording glutamate-evoked transporter currents. Six to 24 h after global ischemia, maximal amplitude of glutamate transporter currents recorded from postischemic CA1 astrocytes was significantly reduced. Western blotting analysis indicated that transient global ischemia decreased the protein level of GLT-1 in the hippocampal CA1 area without affecting GLAST protein level. Further real-time quantitative RT-PCR assays showed that global ischemia resulted in a decrease in GLT-1 mRNA level of hippocampal CA1 region. Global ischemia-induced reduction in GLT-1 expression and glutamate transporter function of CA1 astrocytes precedes the initiation of delayed neuronal death in CA1 pyramidal layer. The present study provides the evidence that transient global ischemia downregulates glutamate transporter function of hippocampal CA1 astrocytes by decreasing mRNA and protein levels of GLT-1.

Neurochemistry International (6)

Astrocytes in culture have been previously shown to express inducible nitric oxide synthase (iNOS) following treatment with cytokines such as interleukin-1[beta] (IL-1[beta]) and interferon-[gamma] (IFN-[gamma]). We report here on the effects of the cyclic nucleotide analogues 8-bromo-cyclic AMP and 8-bromo-cyclic GMP on cytokine-stimulated iNOS gene expression in a cultured murine astrocyte cell line. In these cells, neither 8-bromo-cyclic AMP nor 8-bromo-cyclic GMP alone was able to stimulate iNOS activity. Similarly, neither IL-1[beta] nor IFN-[gamma] was capable of independently stimulating iNOS expression. Co-stimulation with both cytokines, however, resulted in measurable increases in iNOS activity, and correlated to increases in iNOS mRNA levels. The addition of 8-bromo-cyclic AMP, but not 8-bromo-cyclic GMP, was found to further enhance the expression of iNOS activity induced by IL-1[beta] and IFN-[gamma] co-stimulation. This potentiation effect of 8-bromo-cyclic AMP correlated to a further elevation in iNOS mRNA levels over that produced by cytokine co-stimulation alone. However, 8-bromo-cyclic AMP co-treatment with either cytokine alone did not stimulate iNOS activity, indicating that the signal transduction pathway(s) involved in the potentiation effect of 8-bromo-cyclic AMP is functional only in the presence of both cytokines. These results indicate that cyclic AMP-mediated processes can participate in modulating the expression of astrocyte iNOS when the appropriate combinations of stimulatory cytokines are present. (c) 1997 Elsevier Science Ltd. All rights reserved


http://www.sciencedirect.com/science/article/B6T0B-409VHRV-2/2/662ae57b98425516559fdebe84fecc7e

Coagulation Factor XIII (F. VIII), a member of the transglutaminase (TGase) superfamily, is activated by thrombin, cross-links fibrin and stabilizes clots. Another member of this family, tissue TGase (tTG), having similar enzymatic activity, is implicated in neural development and synapse stabilization. Our previous studies indicated that synapse formation and maintenance at the neuromuscular junction (NMJ) involved components of the coagulation cascade in development. Others then showed that either F. XIII or tTG were localized at NMJs in a developmentally-regulated fashion. In the current studies, we addressed the temporal course of skeletal muscle tTG gene expression and found maximal expression at birth and continuing into the immediate postnatal period. Subcellular fractionation revealed a relatively constant particulate isoform of TGase activity which predominated in early embryonic muscle development. In contrast, cytosolic TGase specific activity became the major isoform in the postnatal period. The timing of muscle TGase activity correlated well with expression of tTG mRNA and we now present novel data of Tgm 2 gene expression for tTG in skeletal muscle. Confirming and extending the previous studies, TGase becomes localized at NMJs in the early, further ramifying in the late, neonatal period. These data suggest that the early pulse of particulate activity could coincide with the period of myoblast cell death in embryonic muscle. On the other hand, the peak cytosolic TGase activity occurs in the neonatal period, correlating temporally with muscle prothrombin expression during activity-dependent synapse elimination and possibly the source of the enzyme localized to the NMJ extracellular matrix resulting in synaptic stabilization.

The repeated intracerebroventricular administration of nerve growth factor (5 \(\mu\)g/2.5 \(\mu\)l) to neonatal rats induced the activation of choline acetyltransferase in forebrain cholinergic neurons that was paralleled by a concomitant change in the density of muscarinic cholinergic receptors in the cerebral cortex. The administration of nerve growth factor altered muscarinic binding sites in a biphasic fashion during postnatal development. A significant stimulation of the developmental increase in the density of muscarinic binding sites occurred in nerve growth factor-treated animals at days 2 and 3 after birth. Conversely, nerve growth factor induced a significant decrease in the receptor number at postnatal days 8 and 14. Muscarinic receptor number returned to control values after treatment, suggesting that nerve growth factor-induced changes to muscarinic cholinergic receptors are reversible. Nerve growth factor administration did not affect muscarinic cholinergic receptor density in striatal membranes and did not alter the relative content of cortical messenger RNAs encoding m1 and m3 muscarinic cholinergic receptor subtypes at postnatal day 14, as determined by reverse transcriptase-polymerase chain reaction. The up- and down-regulation of muscarinic cholinergic receptors induced by nerve growth factor during postnatal development may be temporally related events associated with concomitant changes in the activity of choline acetyltransferase.


It is known that the nitric oxide (NO)/cGMP pathway affects neuronal development and the expression of the different proteins is developmentally dependent in several brain areas. However, so far there are no data on the expression of the proteins involved in this signalling system during the development of the cerebellar granule cell, one of the most widely used models of neuronal development. This study was accordingly designed to analyse the developmental regulation of neuronal nitric oxide synthase (nNOS), soluble guanylyl cyclase subunits ([alpha]1, [alpha]2 and [beta]1) and cGMP-dependent protein kinases (cGK I and cGK II) in cerebellar granule cells through real time-polymerase chain reaction (RT-PCR) and Western blotting. We were able to detect guanylyl cyclase subunits and cGK I and cGK II in cerebellar granule cells at every stage of development examined (cells freshly isolated from 7-day-old rat pups, and cells cultured for 7 days or 14 days). Expression levels, nevertheless, varied significantly at each stage. nNOS, [alpha]2 and [beta]1 and cGK II levels increased during granule cell development, while [alpha]1 and cGK I showed an opposite behaviour pattern; the levels of these latter proteins diminished as the cells matured. The functionality of this pathway was assessed by stimulating cells kept in culture for 7 days with DEA/NO or with N-methyl--aspartate (NMDA). Cells responded by increasing intracellular cGMP and activating cGMP-dependent protein kinase activity, which effectively phosphorylated two well-known substrates of this activity, the vasodilator stimulated phosphoprotein (VASP) and the cAMP response element binding protein (CREB). In summary, through both functional and biochemical tests, this is the first demonstration of a complete NO/cGMP signalling transduction pathway in cerebellar granule cells. Our results also indicate the developmental regulation of the proteins in this system.

Choline-O-acetyltransferase (ChAT) is the enzyme which catalyses the biosynthesis of the neurotransmitter acetylcholine in cholinergic neurons. Here we show that in mouse cholinergic NS-20Y neuroblastoma cells cultured in the presence of either okadaic acid (serine/threonine phosphatases 1 and 2A inhibitor) or KN-62 (CaM kinase inhibitor) ChAT activity and mRNA either increased or decreased as a function of the drug concentration, respectively. After 24 h exposure, okadaic acid exerted a dramatic effect on cell morphology; cells became round and had no more neurites. On the contrary, KN-62 induced a slight morphological differentiation of the cells. The present results suggest that phosphatases 1 and 2A and CaM kinase could mediate regulation of ChAT gene expression.


Specific probes were obtained using PCR cloning from rat brain for the 78 kDa glucose regulated (grp78), inducible 72 kDa (hsp70) as well as constitutive 73 kDa (hsc73) heat shock mRNAs. Grp78 and hsc73 were expressed in normal rat brain whereas hsp70 was not. Subcutaneous injection kainic acid (10 mg/kg) produced seizures and induced all three mRNAs. The induction of grp78 and hsp70 mRNAs occurred within 2 h, peaked between 6-8 h, persisted for 48 h, and returned to control levels by 72 h. Expression of the grp78 and hsp70 mRNAs after focal ischemia progressively increased with occlusion durations from 15-120 min in the cerebral cortex. Though grp78 and hsp70 mRNAs were induced modestly in the striatum by 15 min of ischemia, longer durations of ischemia were characterized by little change in the grp78 mRNA levels and relatively lower levels of hsp70 expression. This result indicates that progressive increases in the duration of ischemia in brain, prior to infarction, may produce proportional increases in transcription of the heat shock genes. However, once the duration of ischemia is long enough to produce infarction, this severely limits the availability of ATP which blocks transcription of the heat shock genes. In conclusion, concurrent induction of the heat shock genes suggests that kainic acid seizures and focal ischemia induce several different stress responses in brain cells caused by denaturation of proteins, changes of protein synthesis, and changes of protein glycosylation.


Allelic variants in the promoter region of the serotonin transporter (5-HTT) gene have been implicated in several psychiatric disorders and personality traits. In particular, two common alleles in a variable repeat sequence of the promoter region (SLC6A4) have been differentially
associated with a display of abnormal levels of anxiety and affective illness in individuals carrying the "s" allele. The aim of this study was to compare the basal cerebral metabolic activity of non-psychiatric subjects in fronto-limbic structures to determine whether differences exist in basal metabolic activity within this functional polymorphism. PET scans with fluorine-18 fluorodeoxyglucose as radiotracer were performed in 71 non-psychiatric subjects previously screened for psychopathology and subsequently genotyped for SLC6A4; PET images were compared with SPM2 according to s/s (n = 27), s/l (n = 25), and l/l (n = 19) groups considering a significance threshold in a priori selected areas of P < 0.001 and an extent threshold [greater-than-or-equal]5 voxels. The analysis showed an effect of interest among the three genotype groups in right anterior cingulate gyrus (ACC), left middle frontal gyrus, and left posterior cingulate gyrus (PCC). Comparison between l/l vs. s/s showed increased metabolism for l/l in left middle frontal gyrus and an increase for s/s in right ACC and left PCC. Comparison between s/s vs. s/l showed an increase for s/s in left PCC and right ACC. Increased basal metabolism in fronto-limbic structures for the s/s group may be conceived as an "overactive metabolic state" of these structures, possibly related to an increased susceptibility for developing an anxiety-depression spectrum disorder.

Neurology (10)


http://www.neurology.org/cgi/content/abstract/62/1/100

A form of autosomal recessive spastic ataxia (ARSACS) has been described in the Charlevoix and Saguenay regions of Quebec. So far a frameshift and a nonsense mutation have been identified in the SACS gene. The authors report a new mutation (1859insC), leading to a frameshift with a premature termination of the gene product sacsin, in two sisters from consanguineous parents. The phenotype is similar to previously described patients with ARSACS.


http://www.neurology.org/cgi/content/abstract/61/6/775

Background: In the setting of severe immunosuppression, the polyomavirus JC (JCV) can cause a lytic infection of oligodendrocytes. This demyelinating disease of the CNS white matter (WM) is called progressive multifocal leukoencephalopathy (PML). JCV has a very narrow host-cell range and productive infection of neurons has never been demonstrated. Patient, methods, and results: An HIV-1-infected patient presented with signs of pyramidal tract and cerebellar dysfunction. Brain MRI revealed T2 hyperintensities in the WM of both frontal lobes and cerebellar atrophy. His disease progressed despite therapy and he died 6 months later. In addition to classic PML findings in the frontal lobe WM, autopsy revealed scattered foci of tissue destruction in the internal granule cell layer (IGCL) of the cerebellum. In these foci, enlarged granule cell neurons identified by the neuronal markers MAP-2 and NeuN reacted with antibodies specific for the polyomavirus VP1 capsid protein. Electron microscopy showed 40 nm viral particles, consistent
with polyomaviruses, in these granule cell neurons. In addition, JCV DNA was detected by PCR after laser capture microdissection of cells from the areas of focal cell loss. Finally, in situ hybridization studies demonstrated that many granule cell neurons were infected with JCV but did not contain viral proteins. Sequence analysis of the JCV regulatory region from cerebellar virions showed a tandem repeat pattern also found in PML lesions of the frontal lobe WM. Conclusion: JCV can productively infect granule cell neurons of the IGCL of the cerebellum. This suggests a role for JCV infection of neurons in cerebellar atrophy occurring in HIV-infected individuals.


http://www.neurology.org/cgi/content/abstract/62/7/1170

Background: Brain aromatase may be neuroprotective by increasing the local estrogen levels in injured neurons. Aromatase is encoded by the CYP19 gene located at 15q21.1, a chromosomal region in linkage disequilibrium (LD) with Alzheimer disease (AD) in this sample. Objective: To investigate whether nine single-nucleotide polymorphisms (SNP) spanning the CYP19 gene were associated with AD. Methods: Three hundred ninety-four patients were compared with 469 nondemented control subjects using single-locus and haplotype approaches. Haplotypes were identified using the expectation/maximization algorithm and latent class analysis, which included additional information on age, sex, and APOE polymorphism. Results: Allelic and genotypic frequencies for three adjacent SNP differed between AD and control groups. Both haplotype approaches identified an approximately 60% increase (p = 0.02) in the risk of AD for one haplotype and similar levels of excess risk irrespective of APOE polymorphism and gender. Conclusion: Genetic variation in the brain aromatase gene may modify the risk for AD.


http://www.neurology.org/cgi/content/abstract/64/6/966

Background: Autosomal dominant optic atrophy (ADOA) is the commonest form of inherited optic neuropathy. Mutations in the OPA1 gene encoding a dynamin-related mitochondrial protein underlie ADOA and may perturb the biogenesis and maintenance of mitochondria. Objective: To investigate the mutation spectrum of the OPA1 gene and assess alterations in mitochondrial content caused by OPA1 mutations. Methods: Sixteen Korean patients with clinically suspected ADOA were studied. The mutation spectrum of the OPA1 gene was analyzed by PCR single-strand conformation polymorphism and sequencing, and mitochondrial DNA (mtDNA) content was quantified by real-time PCR. Results: Eight different mutations were found, including five novel mutations. Quantitative real-time PCR analysis showed excellent linearity and precision for the determination of mtDNA copy numbers. The number of mtDNA copies per cell in patients with OPA1 gene mutations (ages 7 to 40) was significantly lower than those in all normal control subjects (p = 0.037), particularly lower than in normal control subjects ages 10 to 39 (p = 0.022). Conclusion: The mutation spectrum of the OPA1 gene disclosed marked genetic heterogeneity and the mitochondrial DNA content was found to be lower in autosomal dominant optic neuropathy, which provides direct evidence for a pathogenetic role of mutations of the OPA1 gene.

The authors report an Italian family with autosomal-dominant Charcot-Marie-Tooth disease (CMT) in which there were giant axons in the sural nerve biopsy. Linkage to the known CMT2 loci (CMT2A, CMT2B, CMT2D, CMT2F) and mutations in the known CMT2 genes (Cx32, MPZ, NEFL), GAN, NEFM, and CMT1A duplication/HNPP deletion were excluded. This family with CMT and giant axons has a pathologic and genetic entity distinct from classic CMT.


The authors investigated the potential association of human primary CNS non-Hodgkin lymphoma (PCNSL) with polyomavirus in HIV-1 infected and uninfected individuals. Immunohistochemical analysis of CNS biopsies from 19 HIV-negative and 17 HIV-positive patients and PCR analysis of 12 HIV-negative and 14 HIV-positive patients revealed that the lymphomas were uniformly negative for polyomaviruses. The authors conclude that polyomaviruses are unlikely to be related to the pathogenesis of most PCNSL.


Background: Mutations in the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) were recently shown to be responsible for autosomal recessive (AR) demyelinating Charcot-Marie-Tooth disease (CMT) type 4A (CMT4A) as well as AR axonal CMT with vocal cord paralysis. Methods: The coding region of GDAP1 was screened for the presence of mutations in seven families with AR CMT in which the patients were homozygous for markers of the CMT4A locus at chromosome 8q21.1. Results: A nonsense mutation was detected in exon 5 (c.581C>G, S194X), a 1-bp deletion in exon 6 (c.786delG, G262fsX284), and a missense mutation in exon 6 (c.844C>T, R282C). Conclusions: Mutations in GDAP1 are a frequent cause of AR CMT. They result in an early-onset, severe clinical phenotype. The range of nerve conduction velocities (NCV) is variable. Some patients have normal or near normal NCV, suggesting an axonal neuropathy, whereas others have severely slowed NCV compatible with demyelination. The peripheral nerve biopsy findings are equally variable and show features of demyelination and axonal degeneration.


Background: Almost 20 years after its discovery, the prevalence and clinical course of human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM, also known as tropical spastic paraparesis [TSP]) remain poorly defined. Whereas the causative association of HTLV-I and
HAM/TSP is generally recognized, controversy still surrounds the relationship between HTLV-II and HAM/TSP. Methods: The HTLV Outcomes Study (HOST--formerly Retrovirus Epidemiology Donor Study [REDS]) is a prospective cohort study including 160 patients with HTLV-I, 405 patients with HTLV-II, and 799 uninfected controls who have been followed every 2 years since 1990-1992. Clinical outcomes are measured by health interviews and examinations, and blood samples are obtained. Results: Six cases of HTLV-I-associated myelopathy (3.7%, 95% CI 1.4 to 8.0) and four cases of HTLV-II myelopathy (1.0%, 95% CI 0.3 to 2.5) have been diagnosed since the formation of the cohort. There have been no cases of HAM/TSP diagnosed among HTLV-negative subjects (0.0%, 95% CI 0.0 to 0.5). Clinical features of the cases include lower extremity hyperreflexia, variably associated with weakness, spasticity, and bladder dysfunction. Conclusions: Systematic screening of HTLV-infected blood donors reveals a high prevalence of HAM/TSP. The clinical course of HAM/TSP appears highly variable. HTLV-II-associated myelopathy generally presents with milder and more slowly progressive signs and symptoms.


http://www.neurology.org/cgi/content/abstract/63/2/234

Background: Deposition of the (beta)-amyloid peptide (A(beta)) in neuritic plaques is a hallmark of Alzheimer disease (AD). Mutations in genes encoding amyloid precursor protein (APP) and presenilin 1 and 2 (PSEN1, PSEN2) are associated with increased accumulation of A(beta) in neuritic plaques or in the walls of cerebral vessels. Intracerebral hemorrhage occasionally affects patients with AD. Methods: A Finnish family with dementia in four generations and with frequent co-occurrence of dementia and intracerebral hemorrhage was identified. Clinical features of 14 family members with a cognitive decline were evaluated. All exons in genes encoding APP, PSEN1, PSEN2, cystatin C, transthyretin, gelsolin, and ITM2B were sequenced, and an association study of APP was conducted by identification of single-nucleotide polymorphisms. Results: Neuropathologic examination revealed Alzheimer-type changes with A(beta) in neuritic plaques and vessel walls, but the cognitive profile of the patients differed from that in AD, as the visuoconstructive functions and verbal fluency were well preserved even in the moderate stage of the disease. In addition to cognitive decline, five patients had had lobar intracerebral hemorrhages and one was diagnosed with hemosiderin deposits in MRI, suggesting previous cerebral microbleeds. No causative mutations were identified in candidate genes associated with amyloid diseases, but linkage to APP region could not be entirely excluded. Conclusions: The family presents an autosomal dominant form of (beta)-amyloidogenic disease that resembles the Italian, Flemish, and Iowa types of AD. No amyloidogenic mutations were identified, but the role of the APP region could not be entirely excluded.


http://www.neurology.org/cgi/content/abstract/64/3/454

Background: Retroviral involvement in the etiology of sporadic ALS has been suspected for several years since the recognition that both murine and human retroviruses can cause motor neuron disease-like syndromes. In a pilot study, an increased prevalence of a retroviral marker (reverse transcriptase [RT] activity) was demonstrated in the serum of British patients with ALS. The current investigation was designed to confirm and extend these findings in a geographically distinct patient cohort under blinded testing conditions. Methods: A highly sensitive product-enhanced RT assay was employed to test coded sera obtained from 30 American patients with sporadic ALS and from 14 of their blood relatives, 16 of their spouses, and 28 nonrelated,
nonspousal control subjects. Results: Serum RT activity was detected in a higher proportion of ALS patients (47%) than in non-blood-related controls (18%; \( p = 0.008 \)). The prevalence of RT activity in the serum of spousal controls (13%) was similar to that in other non-blood-related controls. Unexpectedly, the prevalence of serum RT activity in blood relatives of ALS patients (43%) approached that in the ALS patients themselves. Conclusions: These results confirm that patients with ALS have a significantly higher prevalence of serum reverse transcriptase (RT) activity than that seen in unrelated control subjects. The finding of a similarly increased prevalence in blood relatives of ALS patients raises the possibility that the observed RT activity might be due to an inherited endogenous retrovirus.

**Neuromuscular Disorders** (17)


http://www.sciencedirect.com/science/article/B6T9T-3RGSWVD-7/2/606ddd9721da672e14185924f166b2e

We report on an Austrian pedigree with autosomal dominant amyotrophic lateral sclerosis (ALS), diagnosed in six patients from two generations. The only surviving clinically affected family member was examined in our ALS clinic. Historical information on other affected individuals was obtained from knowledgeable family members. The mean +/- S.D. age of onset of the disease was 54 +/- 6.9 years, with a range of 43-66 years. The duration of the index patient's disease until death was 8 months. Using single strand conformational polymorphism (SSCP) analysis, we studied the index patient's exons 1, 2 and 4 of the Cu/Zn superoxide dismutase gene (SOD1) on chromosome 21. A variant banding pattern was observed for exon 1. Sequencing studies showed a previously underscribed T to A missense mutation at position 8 in exon 1 of the SOD1 gene. This mutation results in the elimination of an Eco571 restriction site. Whereas the index patient was heterozygous for this restriction site, 50 unrelated healthy controls and an unaffected brother were not. The mutation lies in a region involved in dimer contact in the three-dimensional structure of the SOD1 protein. This region comprises other known sites for ALS-causing mutations.


http://www.sciencedirect.com/science/article/B6T9T-4B1SG79-4/2/edf3469750c0b25aee2953e2da97f4f0

In the course of a mutation search performed by muscle dystrophin transcript analysis in 72 Duchenne and Becker Muscular Dystrophies (DMD/BMD) patients without gross gene defect, we encountered four unrelated cases with additional out-of-frame sequences precisely intercalated between two intact exons of the mature muscle dystrophin mRNA. An in silico search of the whole dystrophin genomic sequence revealed that these inserts correspond to cryptic exons flanked by one strong and one weak consensus splice site and located in the mid-part of large introns (introns 60, 9, 1M, and 62, respectively). In each case we identified an intronic point mutation activating the cryptic donor or acceptor splice site. The patients exhibited a
BMD/intermediate phenotype consistent with the presence of reduced amounts of normally spliced transcript and normal dystrophin. The frequency of this new type of mutation is not negligible (6% of our series of 65 patients with 'small' mutations). It would be missed if the exploration of the DMD gene is exclusively performed on exons and flanking sequences of genomic DNA.


http://www.sciencedirect.com/science/article/B6T9T-44HXJS4-7/2/602253887aae167493fe28e0cfcd8c82

The dystrophin gene that is defective in Duchenne muscular dystrophy shows a complex transcriptional control based on several promoters driving independent cell-type-specific expression of different isoforms. Dystrophin isoforms together with dystroglycan, a transmembrane protein which in turn binds to extracellular matrix, are the core of a complex of proteins, the dystrophin-associated protein (DAP) complex, which also comprises cytoplasmic elements like dystrobrevin. Whereas the molecular organization of DAP complex in muscle is well documented, the composition of a similar complex in the nervous system remains largely unknown. We followed by competitive PCR the expression of DAP complex components during retinoic acid (RA)-induced neuronal differentiation of P19 cells. Transcripts for the full-length dystrophin, Dp427, and the short isoform, Dp71, as well as for [alpha]-dystrobrevin 2 increased in parallel with days in culture after RA stimulation, while dystroglycan, [alpha]-dystrobrevin 1 and 3, and [beta]-dystrobrevin were constitutively expressed. The upregulation of some of the components of the dystrophin complex during neuronal maturation suggests functional flexibility of the complex in the nervous system, where specific associations between different isoforms of DAP complex components could possibly organize distinct DAP complex-like complexes.


Amyotrophic lateral sclerosis (ALS) is more common in men than in women (male to female ratio of approximately 2: 1), suggesting a role for a sex-linked factor in the disease. The recent identification of a mutation of the androgen receptor gene in Kennedy's disease or X-linked bulbospinal neuronopathy, a rare form of progressive lower motor neurone degeneration, also associated with clinical signs of androgen insensitivity, raises the possibility that androgen function may be disturbed in other motor neurone disorders, including ALS. The Kennedy's disease mutation consists of an increased size of a highly polymorphic CAG repeat sequence in the first exon of the androgen receptor gene, coding for a polyglutamine tract. We have analysed this CAG repeat sequence in a large number of patients with typical sporadic ALS and in normal controls, in order to test the hypothesis that this polymorphism of the androgen receptor gene may influence susceptibility for ALS. We report that the distribution of alleles relating to the size of the CAG repeat sequence of the androgen receptor gene is similar in ALS and controls, indicating that polymorphisms of the CAG repeat sequence of the androgen receptor gene play a limited role, if any, in susceptibility to ALS.

http://www.sciencedirect.com/science/article/B6T9T-4834GD8-2V/2/af1ed13117c80e64f29e5cb7e53a925d

X-linked dominant inheritance was suspected in a large family with Charcot-Marie-Tooth disease since no male to male transmission was observed, and since the sensory and motor neuropathy was more severe in males than in females. To test linkage to the dominant X-linked Charcot-Marie-Tooth disease (DCMTX) locus in Xq13, genotypes of 19 affected and 19 unaffected individuals from this family were determined for 4 microsatellite markers. Close linkage to mfd66 (DXS453) was found by bipoint analysis (Zmax = 4.8 at [theta] = 0.00). Multipoint analysis mapped the gene between the androgen receptor and DXYS1. In addition, linkage analysis performed with 11 microsatellite markers, derived from a high density map spanning 16 cM on Xq11-Xq21 revealed 3 new tightly linked loci: afm287zgl (DXS1216), afm261zh5 and afm207zg5 (DXS995). Multipoint analysis localized the DCMTX gene to a 7.5 cM interval between afm123xd4 (DXS988) and afm116xg1 (DXS986). Combined analysis with these new microsatellites provides a powerful tool for carrier detection because of their high informativity and the small genetic distance (< 10 cM) between the markers flanking the gene.


http://www.sciencedirect.com/science/article/B6T9T-497Y5H5-5/2/d1325b3f1301f67b17b1a8fb84bd0664

The aim of this study was to evaluate muscle magnetic resonance imaging findings in patients with congenital muscular dystrophy and Ullrich phenotype. Fifteen children with congenital muscular dystrophy and Ullrich phenotype were included in the study. All patients had collagen VI studies in muscle and, when family structure was informative, linkage studies to the collagen 6 loci. Three of the 15 patients had reduced collagen in muscle. One of the three was from an informative family and linked to one of the collagen 6 loci. Another patient was linked to one of the collagen 6 loci but had normal expression of collagen in muscle. The remaining 11 all had normal collagen expression in muscle. Only two of these 11 were from informative families and linkage to collagen 6 loci was excluded in them. All patients had muscle magnetic resonance imaging of their leg muscles using transverse T1 sequences. With the exception of the two patients in whom linkage to the collagen 6 loci was excluded, the other 13 patients showed the same pattern of selective involvement on magnetic resonance imaging of thigh muscles. This consisted of relative sparing of sartorius, gracilis, adductor longus and rectus. This pattern was also found in the case linked COL6A1/A2 locus but with normal collagen. This finding, and the striking clinical and magnetic resonance imaging concordance between patients with normal and reduced collagen VI in muscle suggest that collagen VI could still be the culprit in several cases with normal collagen expression, or alternatively a primary defect in a protein that closely interacts with collagen VI. Mutation analysis of the collagen 6 genes in cases with normal collagen VI expression is needed to resolve this issue.

Mutations in the myotubularin-related protein 2 gene on chromosome 11q22 are known to cause autosomal recessive Charcot-Marie-Tooth disease with irregularly folded myelin sheaths. We screened the coding region of the myotubularin-related protein 2 gene in a Turkish consanguineous Charcot-Marie-Tooth disease family compatible with linkage to chromosome 11q22. A homozygous cytosine to thymine missense mutation at nucleotide position 847, resulting in an amino acid substitution of arginine to tryptophan at codon 283, was detected in exon 9 of the MTMR2 gene. This is the second homozygous missense mutation associated with recessive Charcot-Marie-Tooth disease with focally folded myelin sheaths.


The severe infantile form of myotubular myopathy is a fatal muscle disease that predominantly affects male infants and is characterized by severe weakness and hypotonia from birth. X-linked myotubular myopathy was found to be associated with mutations in the MTM1 gene in Xq28 encoding the putative tyrosine phosphatase, myotubularin. We screened the MTM1 gene for mutations in seven Japanese patients (six males and one female) who had the diagnosis of severe infantile form of myotubular myopathy. We found five mutations, including three novel mutations based on sequence analysis of RT-PCR fragments covering the entire open reading frame. Two patients (one male and one female), who had similar clinicopathologic features, did not have any mutation in the MTM1 gene open reading frame, suggesting that they may have had an autosomal recessive disease.


A novel form of congenital muscular dystrophy in four unrelated patients is proposed. Congenital hypotonia, markedly increased CK, calf pseudohypertrophy and proximal weakness were common early findings. Two cases were severely affected since infancy and never walked. The phenotypical homogeneity was not very evident until advanced stages of the disease. All the patients showed catastrophic progression of the weakness, severe restrictive respiratory insufficiency, macroGLOSSIA, peculiar extreme amyotrophy of hands and feet, and a round and 'puffy' face. All patients became tetraplegic and required mechanical ventilation. Two cases had signs of mild cardiac involvement. The only non-tracheotomised patient died of respiratory complications. No mental retardation or specific brain abnormalities were observed. All patients showed secondary deficit of laminin [alpha]2 and up-regulation of laminin [alpha]5 in muscle. Expression of [alpha]-dystroglycan was severely reduced in two available muscle samples. The known loci for congenital muscular dystrophies were excluded in the only consanguineous case by linkage analysis. Clinical, immunohistochemical and genetic findings strongly suggest a distinct entity.

http://www.sciencedirect.com/science/article/B6T9T-3VX9862-9/2/ca79e57f136a1269ab20ab8e1945521

Introduction of the myogenic-determination gene MyoD forces non-muscle cell cultures into myogenesis, thereby inducing expression of muscle-specific proteins and facilitating their analysis. In several MyoD-transfected fibroblasts, immunohistochemical detection showed expression of desmin after three days, of titin after five days and of dystrophin after seven days. Cell fusion (myotube formation) could be observed after five days. After nine days a fraction of the cells showed a striated titin pattern, indicating an advanced state of muscle differentiation. Dystrophin (the protein absent in Duchenne Muscular Dystrophy patients) can be detected in MyoD-transfected and differentiated fibroblasts from healthy individuals, and is absent in those of patients. MyoD-transfection increases transcription of the dystrophin gene, facilitating RNA-based mutation detection. Using RNA from MyoD-transfected, differentiated fibroblasts of a deceased patient with an unknown, non-deletion mutation, we were able to identify a CGA->TGA nonsense mutation in the rod domain at basepair 6492 and to establish a rapid mutation specific test for future diagnosis of the mutation in his relatives.


http://www.sciencedirect.com/science/article/B6T9T-43T7K72-3/2/cff4fa066fba2002b5e02c50cdaf711f

Malignant hyperthermia is a pharmacogenetic disorder associated with mutations in Ca2+ regulatory proteins. It manifests as a hypermetabolic crisis triggered by commonly used anesthetics. Malignant hyperthermia susceptibility is a dominantly inherited predisposition to malignant hyperthermia that can be diagnosed by using caffeine/halothane contracture tests. In a multigenerational North American family with a severe form of malignant hyperthermia that has caused four deaths, a novel RYR1 A2350T missense mutation was identified in all individuals testing positive for malignant hyperthermia susceptibility. The same A2350T mutation was identified in an Argentinean family with two known fatal MH reactions. Functional analysis in HEK-293 cells revealed an altered Ca2+ dependence and increased caffeine sensitivity of the expressed mutant protein thus confirming the pathogenic potential of the RYR1 A2350T mutation.


http://www.sciencedirect.com/science/article/B6T9T-40PXMC5-B/2/fcc0d451a44243f4fe076990c9315c88

We report on a 5-year-old boy with clinical and neuroradiological evidence of Leigh syndrome and peripheral neuropathy. Skeletal muscle biopsy showed decreased cytochrome c oxidase stain. Ultrastructurally, the nerve biopsy showed a defect of myelination. Biochemical analyses of
muscle homogenate showed cytochrome c oxidase deficiency (15% residual activity). SURF1 gene analysis identified a novel homozygous nonsense mutation which predicts a truncated surf1 protein.


http://www.sciencedirect.com/science/article/B6T9T-4CHRDCB-4/2/683edecd290e8d628830d15801cc82d7

To study pathways involved in human skeletal myogenesis, we profiled gene expression in human primary myoblast cells derived from three individuals using both oligonucleotide and cDNA microarrays. Following stringent statistical testing (false-positive rate 0.4%), we identified 146 genes differentially expressed over time. Interestingly, 86 of these genes have not been reported to be involved in myogenesis in mouse cell lines. This demonstrates the additional value of human primary cell cultures in the study of muscle differentiation. Many of the identified genes play a role in muscle regeneration, indicating the close relationship of this process with muscle development. In addition, we found overlap with expression profiling studies in muscle from Duchenne muscular dystrophy patients, confirming ongoing muscle regeneration in Duchenne muscular dystrophy. Further study of these genes can bring new insights into the process of muscle differentiation, and they are candidate genes for neuromuscular disorders with an as yet unidentified cause.


http://www.sciencedirect.com/science/article/B6T9T-41GWMXM-2/2/87592970186a77ff526e9310c84798

We report a case of congenital muscular dystrophy with secondary merosin deficiency, structural involvement of the central nervous system and mental retardation in an 8-year-old girl from a consanguineous family. She had early-onset hypotonia, generalized muscle wasting, with weakness especially of the neck muscles, joint contractures, mental retardation and high creatine kinase. Muscle biopsy showed dystrophic changes with partial deficiency of the laminin [alpha]2 chain. Cranial magnetic resonance imaging revealed multiple small cysts in the cerebellum, without cerebral cortical dysplasia or white matter changes. The laminin [alpha]2 chain (6q2), Fukuyama type congenital muscular dystrophy (9q31-q33) and muscle-eye-brain disease (1p32- p34) loci were all excluded by linkage analysis. We suggest that this case represents a new entity in the nosology of congenital muscular dystrophy.


http://www.sciencedirect.com/science/article/B6T9T-41GWMXM-1/2/1d23d78ab1de80cf0a2853eacc4aa28f

We describe four Italian patients (aged 3, 4, 12, and 13 years) affected by a novel autosomal
form of recessive congenital muscular dystrophy. These patients were from three non-consanguineous families and presented an almost identical phenotype. This was characterized by hypotonia at birth, joint contractures associated with severe psychomotor retardation, absent speech, inability to walk and almost no interest in their surroundings. In addition, all patients had a striking enlargement of the calf and quadriceps muscles. Ophthalmologic examination revealed no structural ocular abnormalities in any of the children; one patient had severe myopia. In all cases a magnetic resonance imaging of the brain showed an abnormal posterior cranial fossa with enlargement of the cisterna magna and variable hypoplasia of the vermis of the cerebellum. Abnormality of the white matter was also present in all patients, in the form of patchy signal most evident in the periventricular areas. Serum CK was grossly elevated in all. The muscle biopsy from all cases showed dystrophic changes compatible with congenital muscular dystrophy. Immunofluorescence studies showed mild to moderate partial deficiency of laminin [alpha]2 chain. Linkage analysis in the only informative family excluded the known loci for congenital muscular dystrophy, including laminin [alpha]2 chain on chromosome 6q2, the Fukuyama congenital muscular dystrophy locus on 9q3 and the muscle-eye-brain disease on chromosome 1p3. We propose that this represent a novel severe variant of congenital muscular dystrophy, with associated central nervous system involvement.


http://www.sciencedirect.com/science/article/B6T9T-3WWV9D0-9/2/2f185ec20f26a95200eb1f90327ce23e

The mdx mouse, which carries a nonsense mutation in exon 23 of the dystrophin gene, has been used as an animal model of Duchenne muscular dystrophy to evaluate cell or gene replacement therapies. Despite the mdx mutation, which should preclude the synthesis of a functional dystrophin protein, rare, naturally occurring dystrophin-positive fibres have been observed in mdx muscle tissue. These dystrophin-positive fibres are thought to have arisen from an exon-skipping mechanism, either somatic mutations or alternative splicing. Increasing the frequency of these fibres may offer another therapeutical approach to reduce the severity of Duchenne muscular dystrophy. Antisense oligonucleotides have been shown to block aberrant splicing in the human [beta]-globin gene. We wished to use a similar approach to re-direct normal processing of the dystrophin pre-mRNA and induce specific exon skipping. Antisense 2'-O-methyl-oligoribonucleotides, directed to the 3’ and 5’ splice sites of introns 22 and 23, respectively in the mdx pre-mRNA, were used to transfect myoblast cultures. The 5’ antisense oligonucleotide appeared to efficiently displace factors normally involved in the removal of intron 23 so that exon 23 was also removed during the splicing of the dystrophin pre-mRNA. Approximately 50% of the dystrophin gene mRNAs were missing this exon 6 h after transfection of primary mdx myotubes, with all transcripts showing skipping of exon 23 after 24 h. Deletion of exon 23 does not disrupt the reading frame and should allow the synthesis of a shorter but presumably functional Becker-like dystrophin. Molecular intervention at dystrophin pre-mRNA splicing has the potential to reduce the severity of a Duchenne mutation to the milder Becker phenotype.


http://www.sciencedirect.com/science/article/B6T9T-4834GD8-2R/2/b0f583d277d75e93228ad56ff4ec75af

We have characterized the mutation in a feline model of DMD that selectively eliminates expression of the muscle and Purkinje neuronal dystrophin isoforms. The cortical neuronal
isoform was expressed at a detectable level in skeletal muscle in the absence of the muscle promoter and levels of PCR products representing cortical neuronal-type transcripts in dystrophic muscle were comparable to those of normal feline skeletal muscle. Although localized at the sarcolemma, cortical neuronal dystrophin apparently failed to protect skeletal muscle. Neuronal transcripts could not be amplified from feline heart, indicating that these promoters are not active in this tissue in the cat.

Neuron(31)


http://www.sciencedirect.com/science/article/B6WSS-4C6KNF1-13/2/62a61df890a033305fc03220ef0f8ca2

A cDNA clone encoding a K+ channel polypeptide with 72% amino acid sequence identity to Drosophila Shal was isolated from rat hippocampus. Functional expression of the cDNA in Xenopus oocytes generated 4-amino-pyridine-sensitive K+ channels displaying rapid inactivation kinetics. The fastest component of inactivation was slowed by the deletion of 3 basic residues in the amino-terminal region. Northern blots revealed that the mRNA encoding this K+ channel polypeptide was expressed at a similar level in the brain and in the heart. In situ hybridization revealed that the mRNA encoding this K+ channel appeared concentrated in the hippocampus, dentate gyrus, and habenular nucleus in the brain. Thus, this K+ channel polypeptide is likely to form some of the A-type K+ channels expressed in the mammalian nervous system and heart.


http://www.sciencedirect.com/science/article/B6WSS-4C6CSNN-BR/2/0fa2896a304e83aff07014c1b9ed7bb7

Platelet-activating factor (PAF), an alkylether phospholipid, is produced in the brain when it is subjected to various stimuli. Using a Xenopus oocyte expression system, we obtained evidence for functional PAF receptor mRNA expression in rat brain. The presence of the PAF receptor was confirmed and shown to be quite ubiquitous in the CNS by RNA blot and radioligand binding studies. To investigate the neuronal functions of PAF, intracellular Ca2+ increase elicited by nanomolar PAF application was analyzed in cultured rat hippocampal cells. Fractions of NMDA-responsive cells and non-NMDA-responsive cells were shown to respond to PAF, suggesting a potential role for PAF in the Ca2+ signaling pathway in the hippocampus.


http://www.sciencedirect.com/science/article/B6WSS-4C6KNC8-
Paraneoplastic opsonolus-ataxia, a disorder of motor control, develops in breast or lung cancer patients who harbor an antibody (Ri) that recognizes their tumors and a nuclear neuronal protein of 55 kd. We have characterized a gene, Nova, encoding an antigen recognized by the Ri antibody. Nova encodes a novel, highly conserved protein, homologous to the RNA-binding protein hnRNP K, the yeast splicing protein MER1, and a motif in several retroviral proteases. Northern blot analysis detects Nova transcripts only in brain, and several alternatively spliced forms are present in brain and tumor cells. Nova expression is restricted to the ventral brain stem and spinal cord in E18 mice. Since Nova encodes a target antigen in the motor disorder paraneoplastic opsonolus-ataxia that is expressed in the developing subcortical motor system, it is a likely participant in both the pathogenesis of paraneoplastic opsonolus-ataxia and the developmental biology of the motor system. The homology between Nova and hnRNP K suggests that Nova regulates RNA splicing or metabolism in a specific subset of developing neurons.


Sleep is present in all species where it has been studied, but its functions remain unknown. To investigate what benefits sleep may bring at the cellular level, we profiled gene expression in awake and sleeping rats by using high-density microarrays. We find that ~10% of the transcripts in the cerebral cortex change their expression between day and night and demonstrate that half of them are modulated by sleep and wakefulness independent of time of day. We also show that molecular correlates of sleep are found in the cerebellum, a structure not known for generating sleep rhythms. Finally, we show that different functional categories of genes are selectively associated with sleep and wakefulness. The ~100 known genes whose expression increases during sleep provide molecular support for the proposed involvement of sleep in protein synthesis and neural plasticity and point to a novel role for sleep in membrane trafficking and maintenance.


Summary: A combination of genetic factors and early life events is thought to determine the vulnerability of an individual to develop a complex neurodevelopmental disorder like schizophrenia. Pharmacogenetically selected, apomorphine-susceptible Wistar rats (APO-SUS) display a number of behavioral and pathophysiological features reminiscent of such disorders. Here, we report microarray analyses revealing in APO-SUS rats, relative to their counterpart APO-UNSUS rats, a reduced expression of Aph-1b, a component of the [gamma]-secretase enzyme complex that is involved in multiple (neuro)developmental signaling pathways. The reduced expression is due to a duplication-based genomic rearrangement event resulting in an Aph-1b dosage imbalance. The expression levels of the other [gamma]-secretase components were not affected. However, [gamma]-secretase cleavage activity was significantly changed, and the APO-SUS/-UNSUS Aph-1b genotypes segregated with a number of behavioral phenotypes. Thus, a subtle imbalance in the expression of a single, developmentally important protein may be
sufficient to cause a complex phenotype.


http://www.sciencedirect.com/science/article/B6WSS-41838Y2-G/2/7afca99796c01375fe90995efce47e1c

The molecular mechanisms that regulate growth cone guidance of dendrite outgrowth remain to be elucidated. We hypothesized that mRNA localization in dendritic growth cones and their local protein synthesis may be important for growth cone functioning. The appearance of 23 of 31 growth cone mRNAs was developmentally regulated. Also, alteration of growth cone morphology affected the relative levels of three mRNAs. Finally, using single dendrite transfection, it was shown that local protein synthesis occurs in dendrites and growth cones. A heterogeneous population of mRNAs exists in dendritic growth cones of cultured hippocampal neurons whose relative abundances are developmentally regulated and can vary with changes in growth cone physiology. The demonstration of protein synthesis in growth cones suggests that translation of the localized mRNAs may contribute to regulation of growth cone motility and dendrite outgrowth.


http://www.sciencedirect.com/science/article/B6WSS-4CF6DTR-P/2fac8fb9855432883c955fee3b136133f

In situ hybridization histochemistry reveals localized expression of the nicotinic acetylcholine receptor (nAChR) [alpha]2 subunit mRNA restricted to the lateral spiriform nucleus clus (SpL) of the chick diencephalon. The [alpha]2 nAChR transcripts are not detected in immature SpL neurons at 4.5-5 days of embryonic development. They begin to accumulate in the SpL at embryonic day 11 and increase until the newborn stage. Specific [alpha]2 cDNA amplification by the polymerase chain reaction shows that during this period, the absolute content of [alpha]2 mRNA increases about 20-fold. The expression of the [alpha]2 nAChR gene is thus developmentally regulated and appears concomitant with the entry of cholinergic fibers into the SpL, as demonstrated by choline acetyltransferase immunohistochemistry.


http://www.sciencedirect.com/science/article/B6WSS-4C6CS41-W/2/fde20b272ca3b0ffce0f6600c1d2acb5a

Thrombin, a serine protease of the blood coagulation system, has additional effects on cells in vitro. It is mitogenic for fibroblasts and astrocytes and contributes to the regulation of neurite outgrowth and astrocyte stellation. Until now the expression of thrombin or its precursor prothrombin in tissues other than liver has not been demonstrated conclusively because of difficulty in avoiding serum contamination. Using sensitive mRNA detection methods, we show here that prothrombin is expressed not only in the liver, but also in the brain throughout development. Polymerase chain reaction, Northern, and in situ hybridization studies demonstrate the presence of prothrombin transcripts in the olfactory bulb, the cortex, the cerebellum, and other
regions of the rat and human nervous system, as well as in neural cell lines. These results support an involvement of (pro)thrombin in the regulation of cellular events in the nervous system.


http://www.sciencedirect.com/science/article/B6WSS-4C6CSF1-6P/2/dc136daf38f9434e7189f87433e59c62

The short cytoplasmic peptide segment connecting domains III and IV of voltage-gated sodium channels (III-IV linker) is essential for fast inactivation. To test the functional similarity between the III-IV linker and the potassium channel inactivation particle, we attached the III-IV linker to the amino terminus of a noninactivating potassium channel. This chimeric channel inactivated rapidly and displayed biophysical properties similar to Shaker A-type potassium channels. Recovery from inactivation in the chimeric channels was accelerated by high external potassium, consistent with the idea that potassium ions passing through the channel displaced the III-IV linker inactivation particle. A mutation that completely abolishes fast inactivation in rat brain sodium channels also completely abolished inactivation in the chimera. These results demonstrate that the sodium channel III-IV linker can function as a fast inactivation gate and suggest a functional relationship between the fast inactivation processes of sodium and potassium channels.


http://www.sciencedirect.com/science/article/B6WSS-4C6CSHX-88/2/ac1648c2123aa3db13a52d3b349312a6

[gamma]-Aminobutyric acid (GABA) is the most widely distributed known inhibitory neurotransmitter in the vertebrate brain. GABA also serves regulatory and trophic roles in several other organs, including the pancreas. The brain contains two forms of the GABA synthetic enzyme glutamate decarboxylase (GAD), which differ in molecular size, amino acid sequence, antigenicity, cellular and subcellular location, and interaction with the GAD co-factor pyridoxal phosphate. These forms, GAD65 and GAD67, derive from two genes. The distinctive properties of the two GADS provide a substrate for understanding not only the multiple roles of GABA in the nervous system, but also the autoimmune response to GAD in insulin-dependent diabetes mellitus.


http://www.sciencedirect.com/science/article/B6WSS-4B3MJP2-9/2/42fc51933cc0d10172407bbf5ff13df7

The CNS is thought to develop from self-renewing stem cells that generate neurons, astrocytes, and oligodendrocytes. Other data, however, have suggested that astrocytes and oligodendrocytes are generated from separate progenitor populations. To reconcile these observations, we have prospectively isolated progenitors that do or do not express Olig2, an oligodendrocyte bHLH determination factor. Both Olig2- and Olig2+ progenitors can behave as tripotential CNS stem cells (CNS-SCs) in vitro. Growth in FGF-2 causes induction of Olig2 in the
former population, permitting oligodendrocyte differentiation; extinction of Olig2 in the latter cells
permits astrocyte differentiation. The induction of Olig2 by FGF-2 is mediated, in part, via
endogenous Sonic Hedgehog. These data indicate that clonogenic competence to generate
neurons, astrocytes, and oligodendrocytes reflects a deregulation of dorsoventral patterning
during expansion in vitro, raising the question of whether such triffatent cells actually exist in vivo.

Garbern, J. Y., F. Cambi, et al. (1997). "Proteolipid Protein Is Necessary in Peripheral as Well as Central

http://www.sciencedirect.com/science/article/B6WSS-418392HP2/e32eeb35760fff3d1eaf97c2b64cef1

Alternative products of the proteolipid protein gene (PLP), proteolipid protein (PLP) and DM20,
are ma- jor components of compact myelin in the central nervous system, but quantitatively minor
constituents of Schwann cells. A family with a null allele of PLP has a less severe CNS
phenotype than those with other types of PLP mutations. Moreover, individuals with PLP null
mutations have a demyelinating peripheral neuropathy, not seen with other PLP mutations of
humans or animals. Direct analysis of normal peripheral nerve demonstrates that PLP is localized
to compact myelin. This and the clinical and pathologic observations of the PLP null phenotype
indicate that PLP/DM20 is necessary for proper myelin function both in the central and peripheral
nervous systems.

Hollmann, M., J. Boulter, et al. (1993). "Zinc potentiates agonist-induced currents at certain splice variants
of the NMDA receptor." Neuron 10(5): 943.

http://www.sciencedirect.com/science/article/B6WSS-4C6KNVS-7B2/2b408fdcd49fd5a742861d1ff2a9b4fa

We have determined the gene structure for the NMDA receptor subunit gene NMDAR1. We found
eight splice variants that arise from different combinations of a single 5' terminal exon insertion
and three different 3' terminal exon deletions, relative to NMDAR1. We analyzed the modulation
by Zn2+ of currents through homomeric receptors assembled from these splice variants and
found that, in addition to its well-known inhibitory effect at high concentrations, Zn2+ potentiates
agonist-induced currents at submicromolar concentrations (EC50 = 0.50 [mu]M). This potentiation
is observed only with a subset of NMDAR1 splice variants that show additional differences in
pharmacological properties. Zn2+ potentiation is rapidly reversible, noncompetitive with either
glutamate or glycine, and voltage independent. Zn2+ potentiation is mimicked by Cd2+, Cu2+, and
Ni2+, but not by Mn2+, Co2+, Fe3+, Sn2+, or Hg2+. Our results suggest a possible role for
Zn2+ as a positive modulator of NMDA receptors in certain regions of the brain.

Hollmann, M., C. Maron, et al. (1994). "N-glycosylation site tagging suggests a three transmembrane
domain topology for the glutamate receptor GluR1." Neuron 13(6): 1331.

http://www.sciencedirect.com/science/article/B6WSS-4C71KX7-2T2/95a1d8430b5b85f206a59ba76abc2dab

We investigated the transmembrane topology of the glutamate receptor GluR1 by introducing N-
glycosylation sites as reporter sites for an extracellular location of the respective site. Our data
show that the N-terminus is extracellular, whereas the C-terminus is intracellular. Most
importantly, we found only three transmembrane domains (designated TMD A, TMD B, and TMD C), which correspond to the previously proposed TMDs I, III, and IV, respectively. Contrary to earlier models, the putative channel-lining hydrophobic domain TMD II does not span the membrane, but either lies in close proximity to the intracellular face of the plasma membrane or loops into the membrane without traversing it. Furthermore, the region between TMDs III and IV, in previous models believed to be intracellular, is an entirely extracellular domain.


http://www.sciencedirect.com/science/article/B6WSS-4C6KNVS-78/2/572a39f842ff8c7e70f315855db37c79

In the course of studying proteins involved in long-term facilitation in Aplysia, we found that 5-HT and cAMP, a second messenger activated by 5-HT, lead to the removal of a set of N-CAM-related cell adhesion molecules (apCAMs) from the surface membrane of sensory neurons by means of receptor-mediated endocytosis. Here we describe that, as part of this coordinated program for endocytosis, 5-HT and CAMP also induce in the sensory neurons an increase in the density of coated pits and coated vesicles and an increase in the expression of the light chain of Aplysia clathrin (apClathrin). The clathrin-related endocytosis seems designed to internalize and redistribute apCAMs and other surface membrane proteins in the sensory neurons, and thus it appears to constitute one of the initial steps in the growth of new synaptic connections that accompanies long-term facilitation.


http://www.sciencedirect.com/science/article/B6WSS-4C6CS5Y-1W/2/81dfb42eb4d74c63b76e0a5b3ad57811

Acetylcholinesterase (AChE) is concentrated at the vertebrate neuromuscular synapse. To determine whether increased transcript levels could underlie this selective accumulation, we employed a quantitative reverse transcription polymerase chain reaction-based assay to determine mRNA copy number in samples as small as single neuromuscular junctions (NMJs) and a microassay to measure ACNE enzyme activity at single synapses. Our results show that ACNE mRNA is an intermediate transcript at NMJs, whereas in noninnervated regions of muscle fibers, ACNE transcripts are either undetectable or rare. In contrast, a-actin transcript levels in the same samples are similar in junctional and extrajunctional regions. However, compared with ACNE enzyme activity and [alpha]-actin mRNA levels, the levels of ACNE transcripts at NMJs are highly variable. These results indicate that AChE mRNA and protein expression are compartmentalized at the vertebrate NMJ and provide a direct approach toward dissecting the molecular events leading from synaptic activation to plastic changes in gene expression at single vertebrate synapses.


http://www.sciencedirect.com/science/article/B6WSS-4C6CS34-8/2/8e50ff0be839294bfaaf9d27e3436d48
By homology screening of a rat brain library, we have isolated cDNAs that encode a novel member of the synaptophysin/connexin channel protein superfamily. The deduced protein, named synaptoporin, displays 58% amino acid identity to synaptophysin, with highly conserved transmembrane segments, but a divergent cytoplasmic tail. Northern blot analysis and PCR amplification of RNA from different rat tissues indicate expression of synaptoporin transcripts in the CNS. Antibodies against a synthetic peptide or a fusion construct encompassing the cytoplasmic tail region of synaptoporin detect a polypeptide of 37 kd that copurifies with small synaptic vesicles. Our data suggest the existence of a family of vesicular channel proteins whose members may be differently distributed among synaptic vesicle subpopulations.


http://www.sciencedirect.com/science/article/B6WSS-44J1095-G/2/9a8fcddc4d6d17b59c240fd038e0f9cbb

RNA granules are a macromolecular structure observed in neurons, where they serve as motile units that translocate mRNAs. Isolated RNA granules are highly enriched in Staufen protein and ultrastructurally contain densely packed clusters of ribosomes. With depolarization, many mRNAs, including those involved in plasticity, rapidly shift from the RNA granule fraction to polysomes. Depolarization reorganizes granules and induces a less compact organization of their ribosomes. RNA granules are not translationally competent, as indicated by the failure to incorporate radioactive amino acids and the absence of eIF4E, 4G, and tRNAs. We concluded that RNA granules are a local storage compartment for mRNAs under translational arrest but are poised for release to actively translated pools. Local release of mRNAs and ribosomes from granules may serve as a macromolecular mechanism linking RNA localization to translation and synaptic plasticity.


http://www.sciencedirect.com/science/article/B6WSS-41FKPNH-R/2/d0eddf92375a1b4cfde779676505cb056

Voltage-gated ion channels undergo slow inactivation during prolonged depolarizations. We investigated the role of a conserved glutamate at the extracellular end of segment 5 (S5) in slow inactivation by mutating it to a cysteine (E418C in Shaker). We could lock the channel in two different conformations by disulfide-linking 418C to two different cysteines, introduced in the Pore-S6 (P-S6) loop. Our results suggest that E418 is normally stabilizing the open conformation of the slow inactivation gate by forming hydrogen bonds with the P-S6 loop. Breaking these bonds allows the P-S6 loop to rotate, which closes the slow inactivation gate. Our results also suggest a mechanism of how the movement of the voltage sensor can induce slow inactivation by destabilizing these bonds.


http://www.sciencedirect.com/science/article/B6WSS-44FC84P-C/2/03ecf63f5881683ffa5bda1660c76d1
Rods and cones contain closely related but distinct G protein-coupled receptors, opsins, which have diverged to meet the differing requirements of night and day vision. Here, we provide evidence for an exception to that rule. Results from immunohistochemistry, spectrophotometry, and single-cell RT-PCR demonstrate that, in the tiger salamander, the green rods and blue-sensitive cones contain the same opsin. In contrast, the two cells express distinct G protein transducin [alpha] subunits: rod [alpha] transducin in green rods and cone [alpha] transducin in blue-sensitive cones. The different transducins do not appear to markedly affect photon sensitivity or response kinetics in the green rod and blue-sensitive cone. This suggests that neither the cell topology or the transducin is sufficient to differentiate the rod and the cone response.


http://www.sciencedirect.com/science/article/B6WSS-418PW08-R/2/57ab5e568da1d0c83c5f89aa25443419

We show that a thermosensitive splicing event in the 3' untranslated region of the mRNA from the period (per) gene plays an important role in how a circadian clock in Drosophila adapts to seasonally cold days (low temperatures and short day lengths). The enhanced splicing of this intron at low temperatures advances the steady state phases of the per mRNA and protein cycles, events that significantly contribute to the preferential daytime activity of flies on cold days. Because the accumulation of PER is also dependent on the photosensitive TIMELESS (TIM) protein, long photoperiods partially counteract the cold-induced advances in the oscillatory mechanism by delaying the daily increases in the levels of TIM. Our findings also indicate that there is a temperature-dependent switch in the molecular logic governing cycles in per mRNA levels.


http://www.sciencedirect.com/science/article/B6WSS-4C6KNYX-8W/2/a0f11587446a3f96aa3e911fd2e78389

Mice homozygous for the spastic mutation (spa) suffer from a complex motor disorder resulting from reduced CNS levels of the adult glycine receptor isoform GlyRA, which is composed of ligand-binding al and structural [beta] polypeptides. The [beta] subunit-encoding gene (Glyrb) was mapped near the spa locus on mouse chromosome 3. In spa/spa mice, aberrant splicing of the [beta] subunit pre-mRNA strikingly diminishes the CNS contents of fulllength transcripts, whereas truncated [beta] subunit mRNAs accumulate. This is a result of exon skipping, which causes translational frameshifts and premature stop codons. Intron 5 of the spa Glyrb gene contains an L1 transposable element that apparently is causal for the aberrant splicing of [beta] subunit transcripts.


http://www.sciencedirect.com/science/article/B6WSS-4C58XVP-B/2/1afee2f9a639ed7f39f4995fa94c42ee2
Kinesin is a microtubule-based motor protein involved in organelle transport in neuronal and nonneuronal cells. Although a single kinesin motor has been thought to serve all cell types, we document here that neurons express a second conventional kinesin heavy chain (nKHC) that is 65% identical in amino acid sequence to the ubiquitously expressed kinesin heavy chain (uKHC). By preparing antibodies which distinguish between the two KHCs, we demonstrate that nKHC is a nucleotide-dependent microtubule-binding protein which partially cofractionates with membrane organelles. Immunolocalization experiments show that nKHC is distributed throughout the CNS but is highly enriched in subsets of neurons. In hippocampal neurons in culture, uKHC is distributed uniformly throughout the neuron, whereas nKHC is selectively concentrated in the cell body. These results demonstrate that mammalian neuronal tissue contains two conventional kinesin motors which may serve distinct functions in microtubule-based transport.


We have cloned a Kv2 potassium channel from squid optic lobe termed sqKv2. Multiple overlapping sqKv2 cDNA clones differed from one another at specific positions by purine transitions. To test whether the purine transitions were generated by RNA editing, we compared a 360 nucleotide genomic sequence with corresponding cDNA sequences (encoding S4-S6) isolated from individual animals and lying on a single gene and exon. cDNA sequences differed from genomic sequence at 17 positions, resulting in 28 unique sequences. There was invariably an adenosine in the genomic sequence and a guanosine in the edited cDNA sequences. Two of the edits altered the rates of channel closure and slow inactivation. These results extend selective RNA editing to invertebrate taxa and represents a novel mechanism for the posttranscriptional modulation of voltage-gated ion channels.


http://www.sciencedirect.com/science/article/B6WSS-4C71M2W-5P/2/24170ad6a7cf011df89897aa88afa383

RNA editing and subunit assembly of ionotropic glutamate receptors (GluRs) were examined in an oligodendrocyte progenitor cell line, CG4, which expresses GluR2-GluR4, GluR6, GluR7, KA1, and KA2. AMPA-evoked currents rapidly desensitize, whereas kainate-evoked currents contain a steady-state component with a nearly linear current-voltage relation and a fast desensitizing component that is inwardly rectifying. The Q/R site is edited >95% to the arginine codon in GluR2(Q607) mRNA, and <5% in GluR6(Q621) mRNA. Immunoprecipitation experiments demonstrate that GluR6 and/or GluR7 subunits assemble with KA2, but not with GluR2-GluR4. These results indicate that oligodendrocyte progenitor cells selectively edit and assemble glutamate receptors into at least two functionally and structurally distinct heteromeric channels.

To investigate the role of the myelin-associated protein Nogo-A on axon sprouting and regeneration in the adult central nervous system (CNS), we generated Nogo-A-deficient mice. Nogo-A knockout (KO) mice were viable, fertile, and not obviously afflicted by major developmental or neurological disturbances. The shorter splice form Nogo-B was strongly upregulated in the CNS. The inhibitory effect of spinal cord extract for growing neurites was decreased in the KO mice. Two weeks following adult dorsal hemisection of the thoracic spinal cord, Nogo-A KO mice displayed more corticospinal tract (CST) fibers growing toward and into the lesion compared to their wild-type littermates. CST fibers caudal to the lesion--regenerating and/or sprouting from spared intact fibers--were also found to be more frequent in Nogo-A-deficient animals.


The extraordinary cellular heterogeneity of the mammalian nervous system has largely hindered the molecular analysis of neuronal identity and diversity. In order to uncover mechanisms involved in neuronal differentiation and diversification, we have monitored the expression profiles of individual neurons and progenitor cells collected from dissociated tissue or captured from intact slices. We demonstrate that this technique provides a sensitive and reproducible representation of the single-cell transcriptome. In the olfactory system, hundreds of transcriptional differences were identified between olfactory progenitors and mature sensory neurons, enabling us to define the large variety of signaling pathways expressed by individual progenitors at a precise developmental stage. Finally, we show that regional differences in gene expression can be predicted from transcriptional analysis of single neuronal precursors isolated by laser capture from defined areas of the developing brain.


K+ channels are major determinants of membrane excitability. Differences in neuronal excitability within the nervous system may arise from differential expression of K+ channel genes, regulated spatially in a cell type-specific manner, or temporally in response to neuronal activity. We have compared the distribution of mRNAs of three K+ channel genes, Kv1.1, Kv1.2, and Kv4.2 in rat brain, and examined activity-dependent changes following treatment with the convulsant drug pentylenetetrazole. Both regional and cell type-specific differences of K+ channel gene expression were found. In addition, seizure activity caused a reduction of Kv1.2 and Kv4.2 mRNAs in the dentate granule cells of the hippocampus, raising the possibility that K+ channel gene regulation may play a role in long-term neuronal plasticity.

expression of prion protein transgenes derived from long incubation period mice." Neuron 7(1): 59.

http://www.sciencedirect.com/science/article/B6WSS-4C6CSHX-85/2/a1cab262f06910589dcda5890474602f

Prolonged incubation times for experimental scrapie in I/LnJ mice are dictated by a dominant gene linked to the prion protein gene (Prn-p). Transgenic mice were analyzed to discriminate between an effect of the I/LnJPrn-pb allele and a distinct incubation time locus designated Prn-i. Paradoxically, 4 independent Prn-pb transgenic mouse lines had scrapie incubation times shorter than nontransgenic controls, instead of the anticipated prolonged incubation periods. Aberrant or over-expression of the Prn-pb transgenes may dictate abbreviated incubation times, masking genuine Prnp/Prn-i congruence; alternatively, a discrete Prn-i gene lies adjacent to Prn-p.


http://www.sciencedirect.com/science/article/B6WSS-4C6KP83-9P/2/26b759d3ad348ca13af245a4177e6da2

To define the cis-acting DNA elements required for rhodopsin expression, we generated lines of transgenic mice carrying sequences upstream of the bovine rhodopsin gene fused to the E. coli [beta]-galactosidase gene (lacZ). Upstream sequences extending from -2174 to +70 bp, from -734 to +70 bp, and from -222 to +70 by direct photoreceptor-specific expression. All three -2174 lines demonstrate a superior-temporal to inferior-nasal gradient of expression across the retina, whereas lines carrying the shorter constructs demonstrate either spatially continuous expression across the retina, discrete clusters of expression, or both. As a complementary approach to defining regulatory elements, we compared DNA sequences 5' of the murine, bovine, and human rhodopsin genes. Significant homology between all three species was found just upstream of the transcription start site and at approximately 1.5 kb upstream.


http://www.sciencedirect.com/science/article/B6WSS-4CWYS0K-7/2/44032b7f27baa26cf6a00e8cdd7b72d5

The ability of immature neurons from chick lumbosacral sympathetic ganglia to proliferate in vitro was used to identify factors that affect neurogenesis. Under serum-free culture conditions, insulin-like growth factor I (IGF-1), IGF-II, or insulin caused an increase in the proportion of cells that incorporated [3H]thymidine. In addition, IGFs also stimulated neurite outgrowth from these immature sympathetic neurons. IGF-I and IGF-11 mRNA was found to be expressed in E7 sympathetic ganglia during the period of neurogenesis. IGF-I was detectable in fibroblasts, whereas IGF-II mRNA was expressed by neurons, glia, and fibroblasts. Elimination of endogenous IGFs by neutralizing antibodies resulted in a reduction of neuron proliferation and neuron number, whereas elevation of IGF levels by treatment with IGF-I increased sympathetic neuron proliferation in vivo. These findings suggest an important role of IGFs for the development of sympathetic neurons and imply a general role of IGFs in the control of neurogenesis and neurite outgrowth.
Neuropeptides (1)


http://www.sciencedirect.com/science/article/B6WNR-46VHJD-1/2/32d5833b5d3f354fa21d73b5b3e7393f

Neuropharmacology (4)


http://www.sciencedirect.com/science/article/B6T0C-3YDFYV0-G/2/bf6ea69e39e06a8bff07960156702bf3

ATP-induced Ca2+ transients were examined in individual PC12 cells of a well defined clone, before and after treatment with nerve growth factor (NGF) to induce a neurone-like phenotype. Using reverse transcriptase PCR these cells were found to express mRNA for several P2 receptors. In undifferentiated cells the ATP-induced Ca2+ response was entirely dependent on Ca2+ influx, could not be mimicked by UTP, [alpha],[beta]-methylene ATP or dibenzoyl ATP or be blocked by pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS). ATP had no significant effect on levels of cyclic AMP or inositol 1,4,5-trisphosphate (InsP3). These results suggest that in undifferentiated PC12 cells ATP mainly acts on a P2X receptor, possibly the P2X4 subtype. After treatment with NGF for 7 days the ATP response was increased and partially sensitive to PPADS. A component of the ATP-induced Ca2+ increase was due to mobilisation of intracellular Ca2+ stores and another to capacitative Ca2+ entry. UTP caused an increase in intracellular Ca2+, and InsP3 formation could be stimulated by ATP and UTP. ATP also caused a small increase in cyclic AMP, but this was abolished in the presence of indomethacin. Thus, after NGF treatment ATP acts partially via a P2Y receptor, possibly the P2Y2 subtype.


http://www.sciencedirect.com/science/article/B6T0C-3T3JC4N-1B/2/d64e8d714a6c329c1348596c57a6e61e

Evolutionary analysis of neurotransmitter receptor systems has previously focused on interspecies differentiation. Recently, emphasis has shifted to intragenic evolution within a single species and the functional relevance associated with intraspecies variations. For example, multiple polymorphisms have been identified within the human dopamine D2 receptor (DRD2) gene, many of which have been used in clinical association studies. In an attempt to evaluate the
intragenic evolution of the DRD2 gene, genotypes from 116 humans were determined using five biallelic markers which reside within a 30 kb span of the DRD2 gene, that are non-polymorphic in other higher order primates. Only seven different haplotypes, out of a theoretical maximum of 32, were present in the study group of 232 chromosomes. Moreover, five of the seven haplotypes accounted for 99% (n=230/232) of the human haplotypes. A phylogenetic tree was generated from the haplotypic data using a maximum parsimony algorithm. The relationship of the haplotypes within the phylogenetic tree is consistent with a progressive step-wise nucleotide conversion within the human gene. These data indicate that specific haplotypic subtypes of the human DRD2 gene exist within the human population and allow for the possibility that functional differences may exist between the DRD2 subtypes. Therefore, future studies focused on a functional analysis of the entire human DRD2 haplotype, as opposed to individual polymorphisms, may provide important insights into the functional relevance of molecular variations within the human DRD2 gene.


http://www.sciencedirect.com/science/article/B6T0C-48SBTF0-5/2/73526943b68a17bc83a3bd2ced635b25

Previously, a missense polymorphism was identified in the mouse nicotinic receptor [alpha]4 subunit gene, Chrna4. This polymorphism leads to an Ala/Thr variation at amino acid position 529 of the [alpha]4 subunit. Chrna4 A529T is associated with several measures of acute sensitivity to nicotine as well as with mouse strain differences in nicotine-stimulated 86Rb+ efflux from synaptosomes. Here, we report that the variant forms of the mouse [alpha]4 subunit confer functional differences when expressed with the [beta]2 subunit in a heterologous system. [alpha]4[beta]2 receptors containing the T529 variant of the [alpha]4 subunit exhibited a higher EC50 value for the high affinity receptor population and an apparent reduced sensitivity to blockade by DH[beta]E relative to [alpha]4[beta]2 receptors containing the A529 variant of the [alpha]4 subunit. Moreover, the proportion of the total agonist-elicited current contributed by the high affinity [alpha]4[beta]2 receptor population was greater for [alpha]4[beta]2 receptors containing the [alpha]4(T529) variant (64%) than the [alpha]4[beta]2 receptors containing the [alpha]4(A529) variant (41%). These data suggest that the polymorphism in the mouse [alpha]4 subunit is located in a previously unidentified functional domain of the receptor subunit that influences receptor function, including regulation of the affinity population ratio of [alpha]4[beta]2 receptors.


http://www.sciencedirect.com/science/article/B6T0C-4967C3C-3/2/098c4bf023bcb3db69a6a3241ac8fc26

The main aim of this study was to investigate whether intraocular injection of low concentrations of zinc (no greater than 10 [mu]M) aid the survival of ganglion cells in the rat retina after excitotoxic (NMDA) and ischemia/reperfusion injuries. We also determined whether low amounts of zinc cause any detectable retinal toxicity. Intraocular injection of NMDA caused substantial reductions in the mRNA levels of the ganglion cell-specific markers Thy-1 and neurofilament light (NF-L). Co-injection of 0.1 or 1 nmol zinc neither diminished nor exacerbated the effect of NMDA on the levels of these mRNAs. Likewise, ischemia/reperfusion caused significant decreases in the levels of Thy-1 and NF-L mRNAs and in the b-wave amplitude of the electroretinogram. These effects were not counteracted by injection of zinc. Intraocular injection of NMDA caused marked
toxicological effects in retinal glial cells, including upregulations of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), glial fibrillary acidic protein (GFAP), basic fibroblast growth factor (FGF-2) and ciliary neurotrophic factor (CNTF). Interestingly, injection of 1 nmol zinc caused no changes in the levels of COX-2 and iNOS, yet produced similar, although quantitatively less pronounced, changes in FGF-2, GFAP and CNTF. The upregulations of FGF-2 and CNTF suggest that increasing zinc intake may benefit injured retinal neurons. However, this was not found to be the case in the present studies, perhaps due to the acute nature of the injury paradigms utilised.

Neuropsychopharmacology  (3)


http://www.sciencedirect.com/science/article/B6T9V-42WG3XJ-6/2/f9cafc9dc75e04c636e6960d21cb0b

The mechanisms underlying the therapeutic effects of lithium are largely unknown but may involve progressive adaptive alterations at the level of gene expression. Using differential display PCR, we identify a novel cDNA fragment, the expression of which was increased in the rat frontal cortex after 5 weeks of lithium administration. A full-length cDNA (2954-nt) was cloned by arrayed cDNA library screening, and sequencing of the clone revealed an open reading frame of 537-bp encoding a 179-residue protein. Amino acid sequence comparisons revealed that our clone is a member of the Nudix hydrolase family, with the highest percentage of homology (95%) being with a subtype of human diphosphoinositol polyphosphate phosphohydrolase, hDIPP2. Northern blot analysis revealed that chronic lithium treatment significantly increased rDIPP2 mRNA levels in frontal cortex, but not in hippocampus, midbrain, and cerebellum. The effect of lithium on rDIPP2 mRNA expression was not shared by two other anticonvulsant mood stabilizers, carbamazepine and valproate. Time-course studies showed that 1-week of lithium had no effect on rDIPP2 mRNA abundance in the frontal cortex. Our results suggest that DIPP2 may represent a biologically relevant target of lithium therapy, further supporting the notion that abnormalities in inositol phosphate metabolism may be significant in the pathophysiology and pharmacotherapy of bipolar disorder.


http://www.sciencedirect.com/science/article/B6T9V-3WWV9KP-7/2/8d4d8f17a380f359f4566da715e9147c

Knockout of the 5-HT1B gene in mice results in increased aggression, as well as alcohol and cocaine consumption. Given the clinical association of aggression, suicide, alcoholism, and substance abuse, we studied relationship of psychopathology to the human 5-HT1B receptor gene (N = 178) and postmortem human 5-HT1B receptor binding (N = 96) in the brain. The sample comprised: 71 suicide victims, 107 nonsuicides, 45 with a history of major depression and
79 without, 64 with a history of alcoholism or substance abuse and 60 without, as well as 36 with a history of pathological aggression and 42 without. Single-strand conformational polymorphism (SSCP) analysis and DNA sequencing techniques were used to screen the coding region of the human 5-HT1B receptor gene in genomic DNA isolated from postmortem human brain tissue. Two common polymorphisms were identified in the 5-HT1B receptor gene, involving a silent C to T substitution at nucleotide 129 and a silent G to C substitution at nucleotide 861 of the coding region. These polymorphisms were found with the same frequency in the suicide and the nonsuicide groups and in those with and without a history of major depression, alcoholism, or pathological aggression. The binding indices (Bmax and KD of the 5-HT1B receptor in prefrontal cortex also did not differ in suicides and controls, major depression, alcoholism, and cases with a history of pathological aggression. The C129 or G861 allele had 20% fewer 5-HT1B receptor compared to the 129T or 861C allele. We did not identify a relationship between suicide, major depression, alcoholism, or pathological aggression with 5-HT1B receptor binding indices or genotype.


http://www.sciencedirect.com/science/article/B6T9V-44NM021-1/2/e1a417cf45fb2fiae21bd179bc8e475a

We previously reported that ventral tegmental area (VTA) dopamine neurons are supersensitive to AMPA when recorded three days after discontinuing repeated amphetamine or cocaine administration. By increasing dopamine cell activity, this may contribute to the induction of behavioral sensitization. The goal of this study was to determine if increased sensitivity to AMPA reflects increased AMPA receptor expression in the midbrain. Immunolabeling for GluR1, GluR2, GluR2/3, and GluR4 was quantified by immunohistochemistry with 35S-labeled secondary antibodies in VTA, substantia nigra, and a transitional area. First, rats were treated for five days with saline or amphetamine (5 mg/kg) and killed three or 14 days after the last injection. No significant changes in immunolabeling were observed for any subunit at either withdrawal time. GluR1 immunolabeling was further examined in rats killed 16-18 hrs or 24 hrs after a single injection of amphetamine or repeated injections of saline, amphetamine (5 mg/kg x 5 days) or cocaine (20 mg/kg x 7 days). No significant differences were observed in any region. Finally, neither repeated amphetamine or cocaine administration significantly altered GluR1 mRNA levels as quantified by reverse transcriptase-polymerase chain reaction. Our results suggest that enhanced responsiveness of VTA dopamine neurons to AMPA after withdrawal from repeated stimulant administration involves mechanisms more complex than increased expression of AMPA receptor subunits.

Neuroscience (37)


http://www.sciencedirect.com/science/article/B6T0F-3WKXYXY-
The proteins munc18-1 and DOC2 are assumed to play a role in docking of synaptic vesicles in neurotransmitter exocytosis at the presynaptic junction. As the proteins are known to interact, they should co-exist within neurons. We have tested this hypothesis for exocytosis of both classical and peptidergic messengers, by investigating the distribution of the messenger RNAs of munc18-1 and DOC2 homologues in the brain and pituitary gland of the clawed toad Xenopus laevis, using in situ hybridization. For this purpose we cloned a partial complementary DNA encoding Xenopus unc18 (xunc18) and used a corresponding RNA probe, together with an RNA probe for Xenopus DOC2. At the messenger RNA level DOC2 and xunc18 were found to be expressed throughout the Xenopus brain. All brain nuclei expressing DOC2-messenger RNA showed xunc18-messenger RNA expression as well. Co-expression was shown at the individual cell level in consecutive sections of large-sized neurons. A strong expression was demonstrated in the suprachiasmatic and magnocellular nuclei and in peptidergic endocrine cells in the intermediate and anterior lobes of the pituitary gland, suggesting roles of DOC2 and xunc18 in messenger release from peptidergic secretory systems. Combined in situ hybridization and immunocytochemical analyses show that neuropeptide Y-containing cells in the suprachiasmatic nucleus also express DOC2 and xunc18 messenger RNAs. Since these cells have a high secretory activity, controlling the activity of the pituitary pars intermedia, the levels of expression of DOC2 and xunc18 may be indicators for neuronal secretory activity. The present data represent the first evidence for the co-existence of DOC2 and munc18-1 and suggest co-ordinate action of these proteins at the level of brain nuclei, individual neurons and endocrine cells.


http://www.sciencedirect.com/science/article/B6T0F-3VXNH5-W/2/167a8ab62f33da5e84fabac03f9fc3ca

Several recent studies have shown that the ciliary neurotrophic factor exerts myotrophic effects in addition to its well-characterized neurotrophic actions on various neuronal populations. Since expression of acetylcholinesterase in skeletal muscle has been shown to be regulated by putative yet unknown nerve-derived trophic factors, we tested the hypothesis that the ciliary neurotrophic factor is a neurotrophic agent capable of influencing expression of acetylcholinesterase in adult rat skeletal muscle in vivo. To this end, we first determined the impact of daily ciliary neurotrophic factor administration on expression of acetylcholinesterase in both intact and denervated rat soleus muscles. The results of our experiments indicate that although chronic administration of ciliary neurotrophic factor partially counteracted the atrophic response of soleus muscles to surgical denervation, thus confirming its myotrophic effects, it failed to either increase acetylcholinesterase expression in intact muscles or prevent the decrease normally occurring in seven-day denervated muscles. In fact, acetylcholinesterase messenger RNA and enzyme levels were further reduced by ciliary neurotrophic factor treatment in denervated muscles without significant modifications in the pattern of acetylcholinesterase molecular forms. Conversely, transcript levels of the s subunit of the acetylcholine receptor in intact and denervated soleus muscles treated with the ciliary neurotrophic factor were similar to those observed in their respective counterparts from vehicle-treated animals. In addition, we also determined whether transcripts encoding the receptor for the ciliary neurotrophic factor selectively accumulate in junctional domains of rat skeletal muscle fibres. In contrast to the preferential localization of transcripts encoding acetylcholinesterase and the [epsiv] subunit of the acetylcholine receptor within the postsynaptic sarcoplasm, messenger RNAs for the ciliary neurotrophic factor receptor appeared homogeneously distributed between junctional and extra-junctional compartments of both diaphragm and extensor digitorum longus muscle fibres, with no compelling evidence for a selective accumulation within the postsynaptic sarcoplasm. These data show that the ciliary...
neurotrophic factor exerts an inhibitory influence on expression of acetylcholinesterase in muscle fibres. Furthermore, the lack of an effect on expression of the \[\text{epsiv}\] acetylcholine receptor transcripts indicates that treatment with ciliary neurotrophic factor does not lead to general adaptations in the expression of all synaptic proteins. Given the distribution of transcripts encoding the ciliary neurotrophic factor receptor along multinucleated muscle fibres, we propose a model whereby the ciliary neurotrophic factor, or a related unknown molecule that also utilizes the receptor for the ciliary neurotrophic factor, contributes to the maintenance of low levels of enzyme activity in extra-junctional regions of muscle fibres by acting as a repressor of acetylcholinesterase expression that functions directly or indirectly via a pretranslational regulatory mechanism. Accordingly, these results further highlight the complexity of the regulatory mechanisms presiding over acetylcholinesterase expression in vivo.


http://www.sciencedirect.com/science/article/B6T0F-45Y6RG0-19/2/f3034fe5fb1b4ebba9c0fd59d62e7aa

It has become apparent that galanin as well as proopiomelanocortin-derived peptides, such as \[\text{beta}\]-endorphin, play an important role in the hypothalamic circuitry that regulates neuroendocrine functions and appetite behavior. We have recently shown that GalR1 and GalR2 galanin receptor mRNAs are expressed in proopiomelanocortin neurons of the arcuate nucleus, suggesting a direct modulatory action of galanin on the proopiomelanocortin neuronal system. In the present study, we investigated the effect of galanin on \[\text{beta}\]-endorphin release and proopiomelanocortin mRNA expression from male rat mediobasal hypothalamic fragments incubated ex vivo. Galanin induced a decrease of spontaneous \[\text{beta}\]-endorphin release within the first 30-60 min of incubation and this effect was blocked by the galanin receptor antagonist galantide. Co-incubation of galanin with FK-506 (tacrolimus), a calcineurin inhibitor, suppressed the inhibitory effect of galanin on \[\text{beta}\]-endorphin release, suggesting that calcineurin is involved in the galanin-evoked decrease in \[\text{beta}\]-endorphin release. Measurement of \[\text{beta}\]-endorphin levels in the tissues at the end of the incubation period (120 min) revealed that galanin caused a two-fold increase of \[\text{beta}\]-endorphin peptide concentration in the mediobasal hypothalamic tissues. Concurrently, galanin induced an increase in the mean density of silver grains overlying proopiomelanocortin neurons after 60 min of incubation, an effect antagonized by galantide. Finally, reverse transcription-polymerase chain reaction analysis revealed that the mRNAs for the three galanin receptor subtypes (i.e. GalR1, GalR2, and GalR3) were expressed in the incubated mediobasal hypothalamic fragments. Taken as a whole, our results indicate that galanin plays a modulatory role on proopiomelanocortin neurons and this interrelation contributes to the elucidation of the neural circuitry that controls, among others, gonadotropin-releasing hormone function.


http://www.sciencedirect.com/science/article/B6T0F-497YTGN-1/2/cf7c4ad055c398113e637dd8b0a5843c

The cortical information flow via the perforant path represents a major excitatory projection to the hippocampus. Lesioning this projection leads to massive degeneration and subsequently to reorganization in its termination zones as well as in primary non-affected subfields of the hippocampus. The molecular mechanisms and factors which are involved in the postlesional
events are poorly defined. Using a differential display reverse transcription-polymerase chain reaction (DDRT-PCR) strategy, we located one band which occurred only in control hippocampus lanes and almost disappeared in the lanes of lesioned hippocampi. By sequencing, we identified the corresponding gene as cholecystokinin (CCK). Northern blot analysis confirmed a decreased transcription of CCK after lesion. In situ hybridization analysis was performed for localization and quantification of altered CCK transcription. We noted a significant downregulation of CCK transcription in the hippocampus (20%) and in the contralateral cortex (12%) 1-day after lesion (dal) and an increased signal in the ipsilateral cortex (10.5%). This pattern was altered, showing upregulation of CCK mRNA expression, reaching its highest level of 70% above control levels at 5 dal. In the hippocampus, the control level was reached again at 21 dal, whereas the cortex reached the control level at 10 dal. In comparison, the mRNA transcripts of the receptors CCKA and CCKB remained unchanged. Since CCK-containing neurons are involved in the modulation of pyramidal and granule cell excitability, our data indicate a time course correlation between CCK mRNA expression and postlesional axonal sprouting response in the hippocampus.


http://www.sciencedirect.com/science/article/B6T0F-3TN4YT0-1H/2/7e2db40c46cc10b3755444b4885619bb

The aim of this study was to investigate putative effects of calcitonin gene-related peptide on developing dopaminergic neurons in the ventral mesencephalon. To determine a time-point for a physiological role of calcitonin gene-related peptide in the development of this system, we first investigated calcitonin gene-related peptide messenger RNA expression in the ventral mesencephalon of Wistar rats at embryonic days (E) 11-19. Calcitonin gene-related peptide messenger RNA was not detectable at E11, i.e. prior to the appearance of dopaminergic neurons in this area. From E14 to E19, calcitonin gene-related peptide messenger RNA was expressed in increasing amounts. We therefore investigated the effects of calcitonin gene-related peptide on serum-free cell cultures established from the E14 midbrain floor. Addition of calcitonin gene-related peptide (200 ng/ml) every other day significantly increased neuronal differentiation, including longer tyrosine hydroxylase-positive neurites, enhanced immunoreactivity for growth-associated protein-43 and increased dopaminergic uptake per neuron. These effects were maximal after seven to eight days. Calcitonin gene-related peptide acted synergistically with fibroblast growth factor-2 on these parameters. In contrast to fibroblast growth factor-2, however, calcitonin gene-related peptide did not promote survival of tyrosine hydroxylase-immunoreactive neurons. Lack of calcitonin gene-related peptide expression in the mesencephalon at E11 was paralleled by a lack of effect of calcitonin gene-related peptide on early presumptive dopaminergic neurons in terms of eliciting this phenotype. Our data suggest that calcitonin gene-related peptide may act physiologically as a differentiation-promoting factor for phenotypically defined dopaminergic neurons during a time period when dopaminergic neurons assemble in the ventral mesencephalon and grow axons towards their targets.


http://www.sciencedirect.com/science/article/B6T0F-4FDMYB3-4/2/7824b58cebb7df0d9017755894a31a00

Intrathecal (IT) delivery of antisense oligodeoxynucleotides (ASO) has been used to study the function of specific gene products in spinal nociception. However, a lack of systematic studies on the spinal distribution and kinetics of IT ASO is a major hurdle to the utilization of this technique.
In the present study, we injected rats IT with 2'-O-(2-methoxyethyl) modified phosphorothioate ASO (2'-O-MOE ASO) and examined anatomical and cellular location of the ASO in the spinal cord and dorsal root ganglia (DRG) by immunocytochemistry. At 0.5 h after a single IT injection, immunostaining for ISIS 13920 (a 2'-O-MOE ASO targeting h-ras) localized superficially in the lumbar spinal cord, while at 24 h the immunostaining was distributed throughout the spinal cord and was predominantly intracellular. Double staining with cell type specific antibodies indicated that the ASO was taken up by both glia and neurons. ASO immunoreactivity was also observed in DRG after IT ISIS 13920. Capillary gel electrophoresis analysis showed that ISIS 22703, a 2'-O-MOE ASO targeting the [alpha] isozyme of protein kinase C (PKC), remained intact in spinal cord tissue and cerebrospinal fluid up to 24 h after the injection and no metabolites were detected. In contrast, after IT ISIS 11300, an unmodified phosphorothioate ASO with the same sequence as ISIS 22703, no full-length compound was detectable at 24 h, and metabolites were seen as early as 0.5 h. IT treatment with ISIS 22703 at doses that effectively down-regulated PKC[alpha] mRNA in spinal cord did not affect the mRNA expression in DRG. In summary, 2'-O-MOE ASO displayed high stability in spinal tissue after IT delivery, efficiently distributed to spinal cord, and internalized into both neuronal and non-neuronal cells. ASO are able to reach DRG after IT delivery; however, higher doses may be required to reduce target gene in DRG as compared with spinal cord.


http://www.sciencedirect.com/science/article/B6T0F-416BVJ9-G/2/01856ed5e168001d63c775b3dbeab972

GABAB receptors are G-protein-coupled receptors that mediate the slow and prolonged synaptic actions of GABA in the CNS via the modulation of ion channels. Unusually, GABAB receptors form functional heterodimers composed of GABAB1 and GABAB2 subunits. The GABAB1 subunit is essential for ligand binding, whereas the GABAB2 subunit is essential for functional expression of the receptor dimer at the cell surface. We have used real-time reverse transcriptase-polymerase chain reaction to analyse expression levels of these subunits, and their associated splice variants, in the CNS and peripheral tissues of human and rat. GABAB1 subunit splice variants were expressed throughout the CNS and peripheral tissues, whereas surprisingly GABAB2 subunit splice variants were neural specific. Using novel antisera specific to individual GABAB receptor subunits, we have confirmed these findings at the protein level. Analysis by immunoblotting demonstrated the presence of the GABAB1 subunit, but not the GABAB2 subunit, in uterus and spleen. Furthermore, we have shown the first immunocytochemical analysis of the GABAB2 subunit in the brain and spinal cord using a GABAB2-specific antibody. We have, therefore, identified areas of non-overlap between GABAB1 and GABAB2 subunit expression in tissues known to contain functional GABAB receptors. Such areas are of interest as they may well contain novel GABAB receptor subunit isoforms, expression of which would enable the GABAB1 subunit to reach the cell surface and form functional GABAB receptors.


http://www.sciencedirect.com/science/article/B6T0F-4991NC7-1/2/bfc5d4fcd9c069033c42a3bfc0c32f13

Several recent epidemiological studies have proposed that cholesterol-lowering drug Statin may provide protection against Alzheimer's disease (AD). Probucol is a non-Statin cholesterol-
lowering drug and a potent inducer of apolipoprotein E (apoE) production in peripheral circulation. A recent clinical study using Probucol in elderly AD subjects revealed a concomitant stabilisation of cognitive symptoms and significant increases in apoE levels in the cerebral spinal fluid in these patients. To gain insight into the mechanisms underlying these effects, we treated a cohort of aged male rats (26-month-old) with oral dose of Probucol for 30 days. Specifically, we examined the effects of Probucol on apoE production and its receptors (low density lipoprotein receptor [LDLR] and low density lipoprotein receptor-related protein [LRP]), astrogial marker of cell damage (glial fibrillary acidic protein [GFAP]), markers of neuronal synaptic plasticity and integrity (synaptosomal associated protein of 25 kDa [SNAP-25] and synaptophysin) as well as cholesterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A reductase [HMGCoAR]) in the hippocampus. We report that Probucol induces the production of apoE and one of its main receptors, LRP, increases HMGCoAr (rate-limiting enzyme in cholesterol synthesis), substantially attenuates age-related increases in glial activation, and induces production of synaptic marker SNAP-25, a molecule commonly associated with synaptogenesis and dendritic remodeling. These findings suggest that Probucol could promote neural and synaptic plasticity to counteract the synaptic deterioration associated with brain aging through an apoE/LRP-mediated system. Consistent with the beneficial effects of other cholesterol-lowering drugs such as the Statin, Probucol could also offers additional benefits based on apoE neurobiology.


http://www.sciencedirect.com/science/article/B6T0F-3XMGMYX-1B/2/c0e7e3e6a29f4ac49d73d9fbdcb137d

Initial experiments to evaluate the in vivo fate(s) of constitutively proliferating subependymal cells determined that, following in vivo labeling of this population by infection with a retrovirus containing a [beta]-galactosidase reporter gene, there was a progressive and eventually complete loss of histochemically [beta]-galactosidase-positive cells within the lateral ventricle subependyma with increasing survival times of up to 28 days after retroviral infection. Subsequent experiments were designed to ascertain the potential contributions of: (i) the migration of subependymal cells away from the forebrain lateral ventricles; and (ii) the down-regulation of the retroviral reporter gene expression. Retroviral lineage tracing experiments demonstrate that a major in vivo fate for constitutively proliferating subependymal cells is their rostral migration away from the walls of the lateral ventricle to the olfactory bulb. Although down-regulation of retroviral reporter gene expression does not contribute to the loss of detection of [beta]-galactosidase-labeled cells from the lateral ventricle subependyma, it does result in an underestimation of the absolute number of retrovirally labeled cells in the olfactory bulb at longer survival times. Furthermore, a temporal decrease in the double labeling of [beta]-galactosidase-labeled cells with [3H]thyminine was observed, indicating that only a subpopulation of the migratory subependymal-derived cells continue to actively proliferate en route to the olfactory bulb. These two events may contribute to the lack of a significant increase in the total number of retrovirally labeled subependymal cells during rostral migration. Evidence from separately published studies suggests that cell death is also an important regulator of the size of the constitutively proliferating subependymal population. In summary, in vivo studies utilizing retroviral reporter gene labeling demonstrate that constitutively proliferating subependymal cells born in the lateral ventricle migrate rostrally to the olfactory bulb. Loss of proliferation potential and retroviral reporter gene down-regulation contribute to the lack of any significant increase in the total number of labeled cells recovered in the olfactory bulb.

Activin and its cognate receptors are expressed during embryogenesis in the rapidly dividing cells of the basal forebrain ventricular zone. This finding prompted us to study the role of activin in regulating neurotransmitter phenotype expression and other aspects of the ventricular zone-derived progenitor cell differentiation. Although virtually ineffective alone, activin co-operated with fibroblast growth factor 2 to induce a rapid tyrosine hydroxylase-immunoreactivity in cultured ventricular zone progenitors. Northern analysis indicated that the increase in tyrosine hydroxylase-immunoreactivity was associated with increased tyrosine hydroxylase gene expression. Activin and fibroblast growth factor 2 action was specific to tyrosine hydroxylase, as it did not induce the expression of choline acetyltransferase, nor enhance the expression of glutamate decarboxylase. Cultures treated with the DNA replication marker bromodeoxyuridine revealed that both proliferating ventricular zone progenitors and their post-mitotic progeny were induced to express tyrosine hydroxylase. In these cultures, activin acted to reduce fibroblast growth factor 2 stimulated mitotic activity. Furthermore, activin permitted neuronal differentiation and survival of the ventricular zone progenitors after three days in vitro. Together these data demonstrate a novel role of activin and fibroblast growth factor 2 in regulating the fate of the embryonic basal forebrain ventricular zone progenitors.


We have investigated the identity and intracellular cascade of responses resulting from activation of the endogenous 5-hydroxytryptamine receptor in the C6 rat glioma cell line. Sequence analysis of reverse transcription-polymerase chain reaction products derived from C6 glioma cell messenger RNA revealed complete homology with a portion of the rat 5-hydroxytryptamine2A receptor. The binding of [3H]ketanserin to cell membranes demonstrated a significant correlation with the 5-hydroxytryptamine2A receptor in rat frontal cortex. On intact cells, 5-hydroxytryptamine stimulated a concentration-dependent increase in phosphatidylinositol turnover and intracellular [Ca2+] mediated by 5-hydroxytryptamine2A receptors. In whole-cell patch-clamp recordings, 5-hydroxytryptamine induced an outward current mediated predominantly by K+ ions (reversal POTTENTIAL = -80 mV). Using caged molecules containing Ca2+ or inositol 1,4,5-trisphosphate in the patch electrode solution, we found that rapid photolytic release of Ca2+ and particularly inositol 1,4,5-trisphosphate within the cytosol induced an outward current with characteristics similar to those seen after application of 5-hydroxytryptamine. Comparison between differentiated and undifferentiated cells revealed significantly higher receptor density and maximal phosphoinositide response to 5-hydroxytryptamine in undifferentiated cells but the associated rise in [Ca2+] and activation of an outward current was observed more frequently in differentiated cells. Prolonged exposure of the cells to 5-hydroxytryptamine led to a decrease in all responses and to the down-regulation of receptor number. We conclude that the rat C6 glioma cell expresses a 5-hydroxytryptamine2A receptor identical to that found in rat brain and that stimulation of the receptor in C6 cells leads to the activation of Ca2+ activated K+ channels via phosphoinositide hydrolysis and subsequent rise in cytosolic Ca2+ ion concentration. However, the contrasting effects of differentiation on receptor number and phosphoinositide response to 5-hydroxytryptamine compared to Ca2+ release and conductance change indicate that a complex relationship exists between the component parts of the receptor-activated cascade.

http://www.sciencedirect.com/science/article/B6T0F-3W4B16B-13/2/41990c02b42ccd082785d0f33462458e

A subset of familial cases of amyotrophic lateral sclerosis are linked to missense mutations in copper/zinc superoxide dismutase type 1. Patients with missense mutations in copper/zinc superoxide dismutase type 1 develop a paralytic disease indistinguishable from sporadic amyotrophic lateral sclerosis through an unknown toxic gain of function. Nitric oxide reacts with the superoxide anion to form the strong oxidant, peroxynitrite, which participates in neuronal injury in a variety of model systems. Peroxynitrite is an alternate substrate for copper/zinc superoxide dismutase type 1, causing catalytic nitration of tyrosine residues in other proteins. Mutations in copper/zinc superoxide dismutase type 1 may disrupt the active site of the enzyme and permit greater access of peroxynitrite to copper, leading to increased nitration by peroxynitrite of critical cellular targets. To investigate whether neuronal-derived nitric oxide plays a role in the pathogenesis of familial amyotrophic lateral sclerosis, we examined the effects of three different nitric oxide synthase inhibitors: a non-selective nitric oxide synthase inhibitor, nitro-arginine methyl ester; a relatively selective inhibitor of neuronal nitric oxide synthase, 7-nitroindazole; and a novel highly selective neuronal nitric oxide synthase inhibitor, AR-R 17,477, in transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 (Gly->Ala at position 93; G93A) containing a high transgene copy number and a low transgene copy number. AR-R 17,477, but not nitro-arginine methyl ester or 7-nitroindazole, significantly prolonged survival in both the high and low transgene transgenic mice. To determine whether neuronal nitric oxide synthase is involved in the pathogenesis resulting from the familial amyotrophic lateral sclerosis copper/zinc superoxide dismutase type 1 mutation, we produced mice with the copper/zinc superoxide dismutase type 1 mutation which lack the neuronal nitric oxide synthase gene. The transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 on neuronal nitric oxide synthase null background do not live significantly longer than transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1. Western blot analysis indicates the presence of two neuronal nitric oxide synthase-like immunoreactive bands in spinal cord homogenates of the neuronal nitric oxide synthase null mice, and residual neuronal nitric oxide synthase catalytic activity (>7%) is detected in the spinal cord of the transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 on neuronal nitric oxide synthase null background. This amount of residual activity probably does not account for lack of protection afforded by the disrupted neuronal nitric oxide synthase gene in the familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 mice. Immunological nitric oxide synthase is not detected in the copper/zinc superoxide dismutase type 1 mutant mice at several different ages, thus excluding immunological nitric oxide synthase as a contributor to the pathogenesis of familial amyotrophic lateral sclerosis. Levels of neuronal nitric oxide synthase as well as Ca2+-dependent nitric oxide synthase catalytic activity in the copper/zinc superoxide dismutase type 1 mutant mice do not differ from wild type mice. Endothelial nitric oxide synthase levels may be decreased in the copper/zinc superoxide dismutase type 1 mutant mice. Together, these results do not support a significant role for neuronal-derived nitric oxide in the pathogenesis of familial amyotrophic lateral sclerosis transgenic mice.


http://www.sciencedirect.com/science/article/B6T0F-44SHC0J-
Astrocytes express a variety of neurotransmitter receptors which render them capable of responding to extracellular stimuli, like ATP. Release of ATP, e.g. after brain injury, may initiate reactive gliosis via stimulation of purinergic P2X and P2Y receptors. In the present study, the expression and cellular localization of P2X receptor subtypes on astrocytes in the nucleus accumbens of rats under normal physiological conditions and after stab wound were investigated. Reverse transcription-polymerase chain reaction (RT-PCR) with specific P2X1-7 primers, and double immunofluorescence with antibodies to glial fibrillary acidic protein (GFAP, a specific marker of fibrous astrocytes) and to different P2X receptor subtypes (P2X1-4, P2X7) were used. The RT-PCR of tissue extracts of the nucleus accumbens of untreated rats revealed the presence of all seven currently known P2X receptor subtype mRNAs indicating the presence of these receptors in this region. A double immunofluorescence approach with confocal laser scanning microscopy showed the localization of P2X2-4 receptor subtypes on GFAP-labelled astrocytes in untreated rats. Labelling for P2X1 and P2X7 receptor subtypes was not found. After mechanical damage all P2X receptor subtypes studied (P2X1-4, P2X7) were observed on the GFAP-labelled reactive astrocytes. A characteristic distribution of the P2X receptors on astrocytic processes and cell bodies as well as an up-regulation of the P2X-immunofluorescence was found. In conclusion, the data show the presence of P2X receptors on rat nucleus accumbens astrocytes and suggest that astrogliosis in vivo is associated with an up-regulation of distinct P2X receptor subtypes.


Motor recovery after unilateral sensorimotor cortex ablation or sham-injury was measured in apolipoprotein E knockout and wild-type mice by testing their abilities to traverse a narrow beam. All mice trained without difficulty. Sham-operated mice performed perfectly regardless of genotype throughout testing. There was no difference in motor scores between lesioned apolipoprotein E knockout and wild-type mice on a first trial 24 h after injury (P>0.05). There was a significant overall effect of lesion on motor performance (two-way repeated measures analysis of variance F1,42=304, PF17,714=58, PF17,714=58, PF17,714=0.33, P=1.00) and no effect of genotype on the final level of motor performance 12 days after the lesion (Kruskal-Wallis H=5.79, P=0.12). These data suggest that motor recovery after unilateral injury to the sensorimotor cortex does not vary with apolipoprotein E genotype.


Members of the death receptor family may play a prominent role in developmental and pathological neuronal cell death. We report the expression of the TR3 and TR7 death receptors in the adult human and rat central nervous system. Whereas expression of TR3 appears to be high in the human cerebellum, with lower levels in other brain regions, robust expression is observed in many regions of the rat brain. We also analysed modulation of death receptor expression in an in vivo rat model of acute stroke. In contrast to tumour necrosis factor receptor 1, Fas and p75NGFR, which all show up-regulation specifically in lesioned cortex of the permanent middle
cerebral artery occlusion model of stroke, TR3 shows a rapid global increase in both lesioned and unlesioned brain. In comparison, the recently described death receptor TR7 shows no change in this model. These data indicate that the death receptors show clear differences in patterns of expression in response to ischaemic injury.


Orexins-A and -B are neuropeptides derived from a single precursor prepro-orexin. The mature peptides are mainly expressed in the lateral hypothalamic and perifornical areas. The orexins have been implicated in the control of arousal and appear to be important messengers in the regulation of food intake. Two receptors for orexins have been characterised so far: orexin-1 and -2 receptors. To gain a further understanding of the biology of orexins, we studied the distribution of the orexin-1 receptor messenger RNA and protein in the rat nervous system. We first assessed the expression profile of the orexin-1 receptor gene (ox-r1) in different regions by using quantitative reverse transcription followed by polymerase chain reaction. Using immunohistochemical techniques, we investigated the distribution of orexin-1 receptor protein in the rat brain using a rabbit affinity-purified polyclonal antiserum raised against an N-terminal peptide. The orexin-1 receptor was widely and strongly expressed in the brain. Thus, immunosignals were observed in the cerebral cortex, basal ganglia, hippocampal formation, and various other subcortical nuclei in the hypothalamus, thalamus, midbrain and reticular formation. In particular, robust immunosignals were present in many hypothalamic and thalamic nuclei, as well as in the locus coeruleus. The distribution of the receptor protein was generally in agreement with the distribution of the receptor messenger RNA in the brain as reported previously by others and confirmed in the present study. In addition, we present in situ hybridisation and immunohistochemical data showing the presence of orexin-1 receptor messenger RNA and protein in the spinal cord and the dorsal root ganglia. Finally, due to the shared anatomical and functional similarities between orexins and melanin-concentrating hormone, we present a comparison between the neuroanatomical distribution of the orexin-1 receptor and melanin-concentrating hormone receptor protein-like immunoreactivities in the rat central nervous system, and discuss some functional implications. In conclusion, our neuroanatomical data are consistent with the biological effects of orexins on food intake and regulation of arousal. In addition, the data suggest other physiological roles for orexins mediated through the orexin-1 receptor.


Protein kinase C isoforms including the [alpha] isozyme have been implicated in morphine tolerance. In the present study, we examined the effect of intrathecal delivery of an antisense oligonucleotide targeting rat protein kinase C[alpha] mRNA on the expression of spinal protein kinase C[alpha] isozyme and spinal morphine tolerance. Continuous intrathecal infusion of rats with morphine produced an increase in paw withdrawal threshold to thermal stimulation on day 1, which disappeared by day 5. On day 6, a bolus intrathecal injection of morphine (a probe dose) produced significantly less analgesia in morphine-infused rats than in saline-infused rats, suggesting tolerance. Intrathecal treatment with the protein kinase C[alpha] antisense concurrent
with spinal morphine infusion not only maintained the analgesic effect of morphine during the 5-day infusion, it also significantly increased responsiveness to the probe morphine dose on day 6. In comparison, the missense used in the same treatment paradigm had no effect. The inhibitory effect of protein kinase C[alpha] antisense on spinal morphine tolerance was dose-dependent, and reversible. Intrathecal treatment with the antisense, but not the missense, in rats decreased expression of spinal protein kinase C[alpha] mRNA and protein, as revealed by real-time quantitative reverse transcription-polymerase chain reaction and western blots. Expression of the [gamma] isozyme was not affected by the oligonucleotides. The antisense also attenuated protein kinase C-mediated phosphorylation in spinal cord. These results demonstrate that selective reduction in the expression of the spinal protein kinase C[alpha] isozyme followed by a decrease of local protein kinase C-mediated phosphorylation will reverse spinal morphine infusion-induced tolerance. This finding is consistent with the view that tolerance produced by morphine infusion is dependent upon an increase in phosphorylation by protein kinase C, and also it emphasizes that the protein kinase C[alpha] isozyme and its activation in spinal cord may specifically participate in the phenomenon of opiate tolerance.


We report the cloning of the zebrafish [beta] subunit of the glycine receptor and compare the anatomical distribution of three glycine receptor subunit constituents in adult zebrafish brain ([alpha]Z1, [alpha]Z2 and [beta]Z) to the expression pattern of homologous receptor subunits ([alpha]1, [alpha]2 and [beta]) in the mammalian adult CNS. Non-radioactive hybridization was used to map the distribution of the [alpha]Z1, [alpha]Z2 and [beta]Z glycine receptor subunit messenger RNAs in the adult zebrafish brain. The anterior-posterior expression gradient found in adult zebrafish brain was similar to that reported in mammalian CNS. However, the glycine receptor transcripts, notably the [alpha]Z1 subunit, were more widely distributed in the anterior regions of the zebrafish than in the adult mammalian brain. The isoform-specific distribution pattern was less regionalized in zebrafish than in the rat mammalian CNS. Nevertheless, there was some regionalization of [alpha]Z1, [alpha]Z2 and [beta]Z transcripts in the diencephalic and mesencephalic nuclei where different sensory and motor centers express either [alpha]Z1/[beta]Z or [alpha]Z2 subunits. In contrast to the widespread distribution of the [beta] subunit in adult mammalian brain, [alpha]Z2 messenger RNA presented the widest expression territory of all three glycine receptor subunits tested. [alpha]Z2 messenger RNA was expressed in the absence of [alpha]Z1 and [beta]Z messenger RNA in the outer nuclear layer of the retina, the inferior olive and the raphe of the medulla oblongata, as well as in the nucleus of Cajal of the medulla spinalis. In contrast, an identified central neuron of the reticular formation, the Mauthner cell, expresses all three glycine receptor subunits ([alpha]Z1, [alpha]Z2 and [beta]Z). This report extends the already described glycine receptor expression in the vertebrate CNS and confirms the importance of glycine-mediated inhibition in spinal cord and brainstem.


The major aim of this study was to elucidate the role of nitric oxide (NO) in the development of
pentylenetetrazole (PTZ)-kindling as an animal model of primary generalized epilepsy. The daily administration of PTZ is associated with an increase in the amount of neuronal nitric oxide synthase (nNOS). NO generation was measured directly by in vivo and ex vivo electron paramagnetic resonance on rodents undergoing progressive convulsions. We found that primary generalized epilepsy is caused by NO induction during the persistent up-regulation of nNOS expression, but that NO induction is not associated with severe generalized seizures following long-term kindling phenomena after PTZ withdrawal. Morphological changes in the brain structure of rats were measured by magnetic resonance imaging during epileptic convulsions induced by repetitive administration of PTZ. Cerebellum volume for kindled rats decreased 20% but not in rats treated with the nNOS inhibitor, 3Br-7NI, suggesting that generation of NO in the cerebellum is related to decrease in cerebellum volume following PTZ-kindling.


A splice variant of choline acetyltransferase mRNA has recently been identified in the pterygopalatine ganglion of rat. An antibody against this variant protein (designated pChAT) was demonstrated to immunolabel peripheral cholinergic neurons. In the present study, we investigated the expression of pChAT in rat brain. Amongst the brain regions examined, magnocellular neurons in the tuberomammillary nucleus of the posterior hypothalamus were immunohistochemically labelled with anti-pChAT antibody, whilst no immunolabelling was detected in cholinergic neurons in the basal forebrain or striatum. RT-PCR analysis confirmed the expression of pChAT mRNA in the posterior hypothalamus. The distribution of pChAT-positive neurons in the tuberomammillary nucleus was compared with that of neurons positive for adenosine deaminase, which is contained in all neurons of this nucleus. After colchicine treatment to inhibit axonal transport of enzyme, virtually all pChAT-positive cells contained adenosine deaminase. Conversely, about 85% of adenosine deaminase-positive cells contained pChAT in the ventral area, whilst 19% of adenosine deaminase-positive cells were pChAT-positive in the dorsal area. Long axonal projections of pChAT-positive cells in the tuberomammillary nucleus were shown by retrograde labelling of these cells after injection of cholera-toxin B subunit into the cerebral cortex. This study demonstrates that a splice variant of choline acetyltransferase is expressed in the tuberomammillary nucleus of rat. The results raise the possibility that some of the known diverse projection areas of this nucleus may have a cholinergic component.


Synaptosomal-associated protein of 25 kDa (SNAP-25) regulates various membrane fusion processes including exocytosis by endocrine and neural cells. To increase our understanding of the occurrence and regulation of SNAP-25 isoforms, we identified and characterized SNAP-25a and SNAP-25b mRNAs in the pituitary gland and brain of the amphibian Xenopus laevis. The proteins are strongly conserved and are resistant to botulinum neurotoxin A but not to botulinum neurotoxin E, as shown by Western blotting. The spatial distribution of the two SNAP-25 isoforms was assessed with in situ hybridization. Both SNAP-25a mRNA and SNAP-25b mRNA reside in
cells in the pituitary distal lobe and, particularly, in the endocrine melanotrope cells in the pituitary intermediate lobe. The melanotrope cells are involved in the background adaptation process of the skin by releasing \( \alpha \)-melanophore-stimulating hormone. Quantitation of the respective in situ hybridization signals in the Xenopus pars intermedia indicated a differential response, SNAP-25b mRNA being more highly expressed in black-adapted animals than SNAP-25a mRNA, and more than in white-adapted toads. This differential upregulation was also studied by real-time reverse transcriptase polymerase chain reaction, showing that in the intermediate pituitary lobe, both isoforms are physiologically controlled by the background light intensity stimulus, but with different intensities; in black-adapted animals SNAP-25b mRNA is upregulated by 3.33 times compared with white-adapted animals, but SNAP-25a only by 1.96 times. As to neural tissue, in situ hybridization showed that both isoforms coexist throughout the brain, sometimes with similar strengths, but in various areas either SNAP-25a mRNA or SNAP-25b mRNA expression is prevalent. It is speculated that each of the SNAP-25 isoforms in the Xenopus pituitary and brain has a distinct function in cellular fusion processes including secretion, and that their occurrence and regulation depend on the type of secreted neurotransmitter/hormone and/or the activity state of the cell.


http://www.sciencedirect.com/science/article/B6T0F-44J6BDD-2/2/632ba88c0273a727351b19db899ff5ef

BTB/POZ proteins can influence the cell cycle and contribute to oncogenesis. Many family members are present in the mammalian CNS. Previous work demonstrated elevated NAC1 mRNA levels in the rat nucleus accumbens in response to cocaine. NAC1 acts like other BTB/POZ proteins that regulate transcription but is unusual because of the absence of identifiable DNA binding domains. cDNAs were isolated encoding two NAC1 isoforms differing by only 27 amino acids (the longer isoform contains 514 amino acids). The mRNAs for both isoforms were simultaneously expressed throughout the rat brain and peripheral tissues. Semi-quantitative reverse transcription-polymerase chain reaction analysis revealed that the mRNA of the longer isoform was more abundant than the mRNA of the shorter isoform. Western blot analysis demonstrated a similar unequal distribution between the isoforms in the CNS. The longer isoform was the more abundant of the two NAC1 proteins and the ratio between them differed throughout the rat brain. The shorter isoform was not detected in most of the examined peripheral tissues, suggesting differences from the CNS in post-transcriptional processing. Both isoforms repressed transcription in H293T cells using a Gal4-luciferase reporter system. However, the shorter isoform did not repress transcription as effectively as the longer isoform. Transfection of different ratios for both isoforms, in order to replicate the relative amounts observed throughout the CNS, supported an interaction between the isoforms. The net effect on transcriptional repression was determined by the ratio of the two NAC1 isoforms. Each isoform exhibited the subnuclear localization that is characteristic of many BTB/POZ proteins. A rapid and transient increase in the level of the shorter isoform occurred in the nucleus accumbens 2 h following a single i.p. cocaine injection. We conclude that the two isoforms of NAC1 may differentially affect neuronal functions, including the regulation of cocaine-induced locomotion.


http://www.sciencedirect.com/science/article/B6T0F-49NRJH5-3/2/ca40b8b33a378cc096a7ea8a34b3ebb0
P2X receptors are non-selective cation channels gated by extracellular ATP and are encoded by a family of seven subunit genes in mammals. These receptors exhibit high permeabilities to calcium and in the mammalian nervous system they have been linked to modulation of neurotransmitter release. Previously, three complementary DNAs (cDNAs) encoding members of the zebrafish gene family have been described. We report here the cloning and characterization of an additional six genes of this family. Sequence analysis of all nine genes suggests that six are orthologs of mammalian genes, two are paralogs of previously described zebrafish subunits, and one remains unclassified. All nine subunits were physically mapped onto the zebrafish genome using radiation hybrid analysis. Of the nine gene products, seven give functional homo-oligomeric receptors when recombinantly expressed in human embryonic kidney cell line 293 cells. In addition, these subunits can form hetero-oligomeric receptors with phenotypes distinct from the parent subunits. Analysis of gene expression patterns was carried out using in situ hybridization, and seven of the nine genes were found to be expressed in embryos at 24 and 48 h post-fertilization. Of the seven that were expressed, six were present in the nervous system and four of these demonstrated considerable overlap in cells present in the sensory nervous system. These results suggest that P2X receptors might play a role in the early development and/or function of the sensory nervous system in vertebrates.


http://www.sciencedirect.com/science/article/B6T0F-41V2NKH-28/2/d57a84725602599a0a8e1c2c317a111e

Substance P, the most abundant neurokinin in the CNS, is a major modulator of the immune system. We have examined the gene expression of substance P and its receptor in human fetal brain microglia. Using reverse transcription-polymerase chain reaction and Southern blotting assay, the four isoforms of preprotachykinin-A gene transcripts ([alpha], [beta], [gamma] and [delta]) were detected in the microglia. The human fetal microglia produced significantly higher levels of endogenous substance P protein (640-850 pg/106 cells) than did human peripheral blood monocyte-derived macrophages (25-50 pg/106 cells), as determined by an enzyme immunoassay. Using immunohistochemical staining with an anti-substance P antibody, cell membrane substance P immunoreactivity was observed. In addition, we identified the presence of messenger RNA for neurokinin-1 receptor, a primary receptor for substance P in human fetal microglia. From these data, we propose that substance P and its receptor are biologically involved in regulating the functions of microglia, and potentially play an important role in host defense of the central nervous system.


http://www.sciencedirect.com/science/article/B6T0F-4712HRS-1/2/72705fa206ee67da603e37f18db0f7e22e

Previous binding studies have suggested the presence of a so far unknown nicotinic acetylcholine receptor subunit in dorsal root ganglia (Pugh et al., 1995). Here, we investigated whether the most recently identified subunit, [alpha]10, and its potential interaction partner, [alpha]9 (Elgoyhen et al., 2001), are expressed in these ganglia. All neurons of rat dorsal root ganglia, but no glial cells, expressed both [alpha]9 and [alpha]10 mRNA in situ hybridization, and exhibited [alpha]10 immunoreactivity using a newly raised antibody. These findings were confirmed by RT-PCR and western blotting. The data show that dorsal root ganglion neurons coexpress [alpha]9 and [alpha]10 nicotinic receptor subunits, thereby providing the first example of neuronal
Enhanced expression of proinflammatory cytokines and chemokines has long been linked to neuronal and glial responses to brain injury. Indeed, inflammation in the brain has been associated with damage that stems from conditions as diverse as infection, multiple sclerosis, trauma, and excitotoxicity. In many of these brain injuries, disruption of the blood-brain barrier (BBB) may allow entry of blood-borne factors that contribute to, or serve as the basis of, brain inflammatory responses. Administration of trimethyltin (TMT) to the rat results in loss of hippocampal neurons and an ensuing gliosis without BBB compromise. We used the TMT damage model to discover the proinflammatory cytokines and chemokines that are expressed in response to neuronal injury. TMT caused pyramidal cell damage within 3 days and a substantial loss of these neurons by 21 days post dosing. Marked microglial activation and astrogliosis were evident over the same time period. The BBB remained intact despite the presence of multiple indicators of TMT-induced neuropathology. TMT caused large increases in whole hippocampal-derived monocyte chemoattractant protein (MCP)-1 mRNA (1000%) by day 3 and in MCP-1 (300%) by day 7. The mRNA levels for tumor necrosis factor (TNF)-[alpha], interleukin (IL)-1[beta] and IL-6, cytokines normally expressed during the earliest stage of inflammation, were not increased up to 21 days post dosing. Lipopolysaccharide, used as a positive control, caused large inductions of cytokine mRNA in liver, as well as an increase in IL-1[beta] in hippocampus, but it did not result in the induction of astrogliosis. The data suggest that enhanced expression of the proinflammatory cytokines, TNF-[alpha], IL-1[beta] and IL-6, is not required for neuronal and glial responses to injury and that MCP-1 may serve a signaling function in the damaged CNS that is distinct from its role in proinflammatory events.
secretoneurin levels, which could be important for the regulation of striatal output pathways.


http://www.sciencedirect.com/science/article/B6T0F-3Y6PT6X-N/2/8f931643749c34525eece2c6235b4003

Partial sciatic nerve injury, a model of neuropathic pain, elicits a variety of neurochemical, electrophysiological and neuroanatomical changes in primary sensory neurons. We have used the technique of messenger RNA differential display to identify genes with altered expression in these neurons which may contribute to the development of aberrant sensation following such peripheral nerve damage. This approach identified 14 distinct complementary DNA clones, representing transcripts with increased ipsilateral expression in L4/5 dorsal root ganglia, two weeks after unilateral partial ligation of the rat sciatic nerve. Both Zucker diabetic fatty rats and their lean counterparts were used in this study but none of the transcripts identified showed an induction that was confined to one of the two groups. The majority of the clones did not show significant sequence similarity to previously reported genes and therefore may represent novel messenger RNA sequences or, alternatively, unknown regions of partially characterised messenger RNAs. Two of the clones represented transcripts for the known proteins muscle LIM protein and acidic epididymal glycoprotein, neither of which had previously been associated with expression in the nervous system. Reverse transcriptase-polymerase chain reaction analysis and in situ hybridization confirmed that the messenger RNA expression of both muscle LIM protein and acidic epididymal glycoprotein was induced in an ipsilateral-specific manner. Their localisations, examined with in situ hybridization in L5 dorsal root ganglia, were limited in each case to a sub-population of neuronal profiles. Those neuronal profiles that demonstrated muscle LIM protein hybridization were distributed across the profile size range, whereas the distribution of acidic epididymal glycoprotein-positive profiles appeared to be skewed towards smaller profiles. The induction of muscle LIM protein and acidic epididymal glycoprotein in dorsal root ganglia may play an important functional role in the adaptive response of primary sensory neurons following partial sciatic nerve injury.


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We examined the distribution of estrogen receptor (ER)-[alpha] and ER-[beta] immunoreactive (ir) cells in the dorsal (DRN) and median/paramedian (MPRN) raphe nuclei in male mice. ER-[alpha] ir neurons were scattered across the three subdivisions (ventral, dorsal, and lateral) of the DRN and the MPRN. Robust ER-[beta] ir cells were observed throughout the raphe nuclei, and were particularly abundant in the ventral and dorsal subdivisions of the DRN. Using dual-label immunocytochemistry for ER-[alpha] or ER-[beta] with tryptophan hydroxylase (TPH), the rate-limiting enzyme for 5-hydroxytryptamine (5-HT) synthesis, over 90% of ER-[beta] ir cells contained TPH-ir in all DRN subdivisions, whereas only 23% of ER-[alpha] ir cells contained TPH. Comparisons of ER-[alpha] knockout ([alpha]ERKO) as well as ER-[beta] knockout ([beta]ERKO) mice with their respective wild-type (WT) littermates revealed that gene disruption of either ER-[alpha] or ER-[beta] did not affect the other ER subtype expression in the raphe nuclei. In situ hybridization histochemistry revealed that there was a small but statistically significant decrease in TPH mRNA expression in the ventral DRN subdivision in [beta]ERKO mice compared with
[beta]WT mice, whereas TPH mRNA levels were not affected in [alpha]ERKO mice. These findings support a hypothesis that ER-[beta] activation may contribute to the estrogenic regulation of neuroendocrine and behavioral functions, in part, by acting directly on 5-HT neurons in the raphe nuclei in male mice.


http://www.sciencedirect.com/science/article/B6T0F-44J73KB-G/2/810874afa3e4e8e045933809ca3d13f7

Neurturin and glial cell line-derived neurotrophic factor are novel mitogens for normal adult rat chromaffin cells in vitro. These neurotrophic factors differ from the previously described adult chromaffin cell mitogens, nerve growth factor and basic fibroblast growth factor, in that their effects are potentiated by depolarization and activation of protein kinase C. Neurturin and glial cell line-derived neurotrophic factor signal via the receptor tyrosine kinase, ret, but may also act independently of ret. Both depolarization and phorbol esters act synergistically with neurturin to up-regulate ret protein expression in chromaffin cell cultures, suggesting a mechanism for potentiation of mitogenesis. However, a direct role for ret in mitogenesis has not been established. Stimulation by neurturin causes increased phosphorylation of extracellular signal-regulated kinases 1 and 2 in cultured chromaffin cells, and mitogenesis is prevented by inhibitors of their phosphorylation. Inhibitors of phosphatidylinositol 3-kinase also prevent mitogenesis. The present findings suggest the hypothesis that neurotrophic factors and neurally derived signals might cooperatively regulate chromaffin cell proliferation in vivo in the rat. In addition, trans-synaptic stimulation might provide a route by which epigenetic factors could influence the development of adrenal medullary hyperplasia in humans with hereditary multiple endocrine neoplasia syndromes 2A and 2B by affecting expression and/or activation of ret.


http://www.sciencedirect.com/science/article/B6T0F-45F4VF7-J/2/cdbad4d48a55dd5c5e17dcff4a8046c8

Effects of the lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid were studied in cultured murine microglia using the patch-clamp and video imaging techniques. Both lysophospholipids induced transient membrane hyperpolarization and K+ current activation. The lysophospholipid-induced K+ current was blocked by charybdotoxin or iberiotoxin, but was unaffected by apamin. In recordings with 1 [mu]M intracellular free Ca2+, Ca2+-dependent K+ currents of microglia showed a similar pharmacological profile to lysophospholipid-induced currents. The Ca2+-dependent K+ channels activated in microglia by lysophospholipids are most likely encoded by the IKCa1 channel gene. The presence of IKCa1 mRNA in microglia was demonstrated by reverse transcriptase-polymerase chain reaction studies. Ca2+ imaging experiments revealed increases in the intracellular free Ca2+ concentration of microglia to a mean value of about 400 nM after application of 1 [mu]M sphingosine-1-phosphate or 1 [mu]M lysophosphatidic acid. We suggest that the transient membrane hyperpolarization seen in microglia following exposure to sphingosine-1-phosphate or lysophosphatidic acid is caused by activation of IKCa1 Ca2+-dependent K+ channels. Increases in the concentration of intracellular free Ca2+ evoked by the lysophospholipids are sufficient to activate microglial Ca2+-dependent K+ channels.

http://www.sciencedirect.com/science/article/B6T0F-3S59W9T-T/2/faa2426def1505d072dc6025680826fc

Substance P is an important neuropeptide neurotransmitter in the central, autonomic and enteric nervous systems. In sympathetic ganglia, substance P is thought to play a role in modulating synaptic transmission. Release of substance P by neuronal stimulation or direct application of substance P to ganglionic neurons increases neuronal excitability. An amphibian substance P receptor complementary DNA has been cloned and characterized from bullfrog, Rana catesbeiana, sympathetic ganglion complementary DNA libraries. The deduced primary structure contains features indicative of a seven transmembrane domain G-protein-coupled receptor. The deduced protein sequence shows 69% identity to previously cloned mammalian substance P receptors. In situ hybridization analysis performed on bullfrog sympathetic ganglia using digoxigenin-labelled complementary RNA probe demonstrated that approximately 75% of the principal neurons displayed reaction product above background levels. Radioligand binding studies were performed on stably transfected cells with [125I]Tyr-1-substance P as the ligand. Substance P had an IC50 of 16 nM and the agonist potency profile was substance P>neurokinin A>>neurokinin B. The order of potency for three tachykinins to increase intracellular calcium when applied to a stably transfected clonal cell line was substance P>neurokinin A>>neurokinin B. This order of agonist potency also held for inhibition of the M-type potassium current in intact bullfrog sympathetic neurons. The non-peptide substance P antagonists CP-96345 and RP-67580 at concentrations that block mammalian substance P receptors had little or no effect on the responses to substance P at the bullfrog receptor. Overall, these results demonstrate that the cloned sequence has the features consistent with and characteristic of a substance P receptor. The results are discussed with reference to the established pharmacology of the bullfrog substance P receptor and known structure-activity relationships of mammalian tachykinin receptors.


http://www.sciencedirect.com/science/article/B6T0F-3S8397P-1H/2/e2d2d9a2e202e5aa6b60c879b490a971

A novel gene, designated neurorep 1, was isolated by differential hybridization screening from a complementary DNA library constructed from the rat facial nucleus whose nerve had been transected seven days before sampling. In situ hybridization revealed that this gene was up-regulated in the repair stage after axotomy. The deduced protein, Neurorep 1, consists of 293 amino acid residues, and its molecular mass is approximately 34,000. Protein sequence motif search indicates that this protein has an ecto-5'-nucleotidase consensus sequence at the carboxyl terminal region. In vitro studies showed that Neurorep 1 significantly increased the activity of ecto-5'-nucleotidase, which is considered to be involved in regeneration and repair of the central nervous system. Neurorep 1 might play a significant role in the repair process of nerve tissues by its regulation of ecto-5'-nucleotidase activity.

patients share the molecular fingerprints to develop in a neoplastic fashion: A microarray analysis study.” Neuroscience 131(2): 359.

http://www.sciencedirect.com/science/article/B6T0F-4F9SXWD-1/2/60f916b073a653218c6c823ed7606aae

Identification of genetic mechanisms that promote the onset of stroke and transient cerebral ischemic attack symptoms in carotid atherosclerotic patients would further our understanding of the pathophysiology of this disease and could lead to new pharmacological and molecular therapies. Using Affymetrix Human Genome 230 GeneChip set, the present study evaluated the gene expression differences in geometrically similar carotid artery plaque samples extricated from six symptomatic stroke patients and four asymptomatic patients. There was no significant difference in the degree of stenosis between the two groups. Of the 44,860 transcripts analyzed, 289 (approximately 0.6% of the total transcripts) were differentially expressed between the plaques from the symptomatic and asymptomatic groups (236 were expressed more abundantly and 53 were expressed less abundantly in the symptomatic group). Of the 236 transcripts expressed more abundantly in the symptomatic plaques, 71% (167 transcripts) indicate an active cell proliferation and neoplastic process. These include oncogenes, growth factors, tumor promoters, tumor markers, angiogenesis promoters, transcription factors, RNA splicing factors, RNA processing proteins, signal transduction mediators and those that control the metabolism. Real-time polymerase chain reaction confirmed the increased expression of 63 transcripts in the symptomatic plaques. The other groups of transcripts expressed more abundantly in the symptomatic plaques are those that control ionic homeostasis, those that participate in the progression of degenerative neurological diseases (Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease) and epilepsy. This indicates that symptomatic plaques are molecularly and biochemically more active than the asymptomatic plaques, or active plaque growth precipitates stroke symptoms.


http://www.sciencedirect.com/science/article/B6T0F-46P9MSK-3/2/ad0393314a74c9ad1c00515e81e1e740

Neuropathic pain is induced by injury or disease of the nervous system. Studies aimed at understanding the molecular pathophysiology of neuropathic pain have so far focused on a few known molecules and signaling pathways in neurons. However, the pathophysiology of neuropathic pain appears to be very complex and remains poorly understood. A global understanding of the molecular mechanisms involved in neuropathic pain is needed for a better understanding of the pathophysiology and treatment of neuropathic pain. Towards this end, we examined global gene expression changes as well as the pathobiology at the cellular level in a spinal nerve ligation neuropathic pain model using DNA microarray, quantitative real-time PCR and immunohistochemistry. We found that the behavioral hypersensitivity that is manifested in the persistent pain state is accompanied by previously undescribed changes in gene expression. In the DRG, we found regulation of: (1) immediate early genes; (2) genes such as ion channels and signaling molecules that contribute to the excitability of neurons; and (3) genes that are indicative of secondary events such as neuroinflammation. In addition, we studied gene regulation in both injured and uninjured DRG by quantitative PCR, and observed differential gene regulation in these two populations of DRGs. Furthermore, we demonstrated unexpected co-regulation of many genes, especially the activation of neuroinflammation markers in both the PNS and CNS. The results of our study provide a new picture of the molecular mechanisms that underlie the complexity of neuropathic pain and suggest that chronic pain shares common pathobiology with progressive neurodegenerative disease.

http://www.sciencedirect.com/science/article/B6T0F-46T45F8-P/2/946922cf5bcb67b6bf1808f136c41d26

Proteolytic fragments generated by ADAMTS (integrin and etallopeptase with hrombopondin motifs)-mediated cleavage of the aggregating chondroitin sulfate proteoglycan, brevican, have been identified, but not localized in the CNS. The purpose of this study, using kainate-induced CNS lesion, was to examine the spatial and quantitative relationship between ADAMTS1 and 4 mRNA expression and ADAMTS-mediated cleavage of brevican (as determined by the abundance of the neo-epitope QEAVESE at the C-terminal of the cleaved brevican G1 domain). In untreated rats, in situ hybridization and reverse transcriptase polymerase chain reaction indicated that ADAMTS4 expression was higher than ADAMTS1 and was localized to hippocampus, temporal lobe and other areas of cortex, striatum and hypothalamus. ADAMTS4 mRNA expression in these regions correlated with the presence of the QEAVESE neo-epitope, which was concentrated in perineuronal nets and in neuropil. In rats that seized after kainate, there was a dramatic elevation in ADAMTS1 and ADAMTS4 transcript that correlated and co-localized with a robust elevation in an extractable, 55-kDa fragment of brevican in temporal lobe and hippocampus. This fragment consisted, at least in part, of the ADAMTS-cleaved epitope G1-QEAVESE. The kainate-induced elevation in this ADAMTS-cleaved fragment was localized to amygdaloid and thalamic nuclei, hippocampus, caudate-putamen, cingulate cortex, and the outer molecular layer of the dentate gyrus where it was accompanied by a robust elevation in ADAMTS1 and 4 mRNA and a 28% decline in synaptic density 5 days after kainate. Thus, complexes of extracellular matrix proteins that exist in perineuronal nets and in neuropil are cleaved by specific matrix-degrading proteases at early time points during excitotoxic neurodegeneration. The observed ADAMTS-induced cleavage of brevican in the dentate outer molecular layer is closely associated with diminished synaptic density, and may, therefore, contribute to synaptic loss and/or reorganization in this region.


http://www.sciencedirect.com/science/article/B6T0F-4684HBY-H/2/4b8382fe0f6fb7282080ee262525246c

We hypothesize that sleep state-dependent withdrawal of serotonin (5-hydroxytryptamine, 5-HT) at upper airway (UAW) dilator motoneurons contributes significantly to sleep-related suppression of dilator muscle activity in obstructive sleep apnea. Identification of 5-HT receptor subtypes involved in postsynaptic facilitation of UAW motoneuron activity may provide pharmacotherapies for this prevalent disorder. We have adapted two assays to provide semi-quantitative measurements of mRNA copy numbers for 5-HT receptor subtypes in single UAW motoneurons. Specifically, soma of 111 hypoglossal (XII) motoneurons in 10 adult male rats were captured using a laser dissection microscope, and then used individually in single round molecular beacon polymerase chain reaction (PCR) for real-time quantitation of 5-HT2A, 5-HT2C, 5-HT3, 5-HT4, 5-HT5A, 5-HT5B, 5-HT6 or 5-HT7 receptor. Receptor mRNA copy numbers from single XII motoneuronal soma assayed for the 5-HT2A receptor had measurable copy numbers (7028+/-2656 copies/cell). In contrast, copy numbers for the 5-HT2A receptor in XII non-motoneuronal (n=17) and lateral medulla (n=15) samples were 81+/-51 copies and 83+/-35
copies, respectively. P2C receptor copy numbers of mRNA (287+/−112 copies/cell). XII soma had minimal 5-HT3, 5-HT4, 5-HT5A, 5-HT5B, 5-HT6 or 5-HT7 receptor mRNA. 5-HT2A receptor mRNA presence within XII motoneurons was confirmed with digoxigenin-labeled in situ hybridization. In summary, combined use of laser dissection and molecular beacon PCR revealed 5-HT2A receptor as the predominant 5-HT receptor mRNA in XII motoneurons, and identified small quantities of 5-HT2C receptor. This information will allow a more complete understanding of serotonergic control of respiratory activity.

Neuroscience & Biobehavioral Reviews (1)


To understand the onset and the molecular mechanisms triggering dopaminergic (DA) dysregulation in Attention-Deficit Hyperactivity Disorder (ADHD), we have used the Spontaneously Hypertensive Rats (SHR), the most widely studied animal model for this disease. We have studied the pattern of expression of specific genes involved in DA neuron differentiation, survival and function during postnatal (P) development of the ventral midbrain in SHR males. Our results show that tyrosine hydroxylase and DA transporter gene expression are significantly and transiently reduced in the SHR midbrain during the first month of postnatal development, although with a different kinetic. The other genes analyzed do not show significant variation between SHR and control rats. In addition, high-affinity DA uptake activity is significantly reduced in synaptosomes obtained from the striatum of 1-month-old SHR, when compared to controls. Our data suggest that down-regulation of DA neurotransmission occurs in the midbrain of SHR in a developmentally regulated temporal window during postnatal development, thus strengthening the hypodopaminergic hypothesis in the pathogenesis of ADHD.

Neuroscience Research (9)


N-Methylated [beta]-carbolines, including 2-methylnorharman, are structural and functional analogs of the parkinsonian-inducing toxin, MPP+. We are investigating N-methylated [beta]-carbolines, including 2-methylnorharman, as possible etiologic factors in the pathogenesis of Parkinson's disease. The cellular targets of N-methylated [beta]-carboline-mediated cytotoxicity
are unknown; therefore, we used the T7Select(R) Phage Display System in a novel approach to identify brain proteins that bind to 2-methylnorharman. We incubated (biopanned) immobilized 2-methylnorharman with a phage display cDNA library that expressed a library of human brain proteins on the surface of bacteriophage T7. We washed off unbound phage, amplified the phage that were bound to 2-methylnorharman, and enriched for toxin-interacting phage by repeating the biopanning and amplification steps. The cDNA sequences from the toxin-interacting phage were used to derive the amino acid sequences of the phage-displayed proteins. Five of the six 2-methylnorharman-interacting proteins may have relevance to Parkinson's disease: [alpha]-tubulin, paraoxonase, dorfin, fatty acid binding protein, and platelet-activating factor acetylhydrolase. Dorfin has sequence homology with parkin, which is interesting because mutations in the parkin gene associate with early-onset Parkinson's disease. Our findings are the basis for future studies aimed at determining whether 2-methylnorharman affects the function of these specific proteins in vitro and in vivo.


http://www.sciencedirect.com/science/article/B6T0H-4DBKHBV-2/2/91d972056b554492493e726e3bddabbf

The system L-amino acid transporter is a major nutrient transport system that is responsible for Na+-independent transport of neutral amino acids including several essential amino acids. We have compared and examined the expressions and functions of the system L-amino acid transporters in both rat astrocyte cultures and C6 glioma cells. The rat astrocyte cultures expressed the L-type amino acid transporter 2 (LAT2) with its subunit 4F2hc, whereas the L-type amino acid transporter 1 (LAT1) was not expressed in these cells. The C6 glioma cells expressed LAT1 but not LAT2 with 4F2hc. The [14C]L-leucine uptakes by the rat astrocyte cultures and C6 glioma cells were Na+-independent and were completely inhibited by the system L selective inhibitor, BCH. These results suggest that the transport of neutral amino acids including several essential amino acids into rat astrocyte cultures and C6 glioma cells are for the most part mediated by LAT2 and LAT1, respectively. Therefore, the rat astrocyte cultures and C6 glioma cells are excellent tools for examining the properties of LAT2 and LAT1, respectively. Moreover, the specific inhibition of LAT1 in cancer cells might be a new rationale for anti-cancer therapy.


http://www.sciencedirect.com/science/article/B6T0H-4CWSWST-1/2/1229c0f97a9f847ebf7526fa315a5ed0

Serotonergic innervation of the central nervous system has a sexual dimorphism. The serotonin level in the hypothalamus was modulated by estrogen, and the formation of sexual dimorphism of serotonergic fiber innervation in the hypothalamus has been shown by the effect of sexual hormone during the critical perinatal period. In this study, we examined the direct effect of estrogen on the neurite growth of serotonergic neurons in primary culture from embryonic day 14 (E14) of rat mesencephalon. The total neurite length of serotonin-immunoreactive (IR) cells was significantly decreased by estradiol benzoate (E2, 10-8 M) treatment for 7 days, compared with the case of no treatment. Moreover, the presence of estrogen receptor (ER) [alpha] and ER[beta] mRNA in the E14 mesencephalon with reverse transcription-polymerase chain reaction (RT-PCR), and the ER[alpha] or ER[beta] protein in the cultured serotonin-IR cells with double fluorescence immunohistochemistry were also demonstrated. Our results suggest that the inhibitory effects of E2 on the neurite growth of serotonergic cells expressing ER[alpha] or
ER$[\beta]$ might be involved in the formation of the sexual dimorphic distribution of serotonergic innervation.


http://www.sciencedirect.com/science/article/B6T0H-42M78PT-6/2/51b8933e1c91ae0bca976e522d9ca3dda

We investigated the neuropathological and biochemical changes in the white matter of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) after bilateral carotid artery ligation (BCAL). One week after BCAL, both WKY and SHR showed white matter rarefaction and vacuolation with reduced oligodendrocytes, but there was no difference between WKY and SHR. On the other hand, vacuoles formed by oligodendroglial cell death were increased significantly from 2 to 4 weeks in the optic tract and fimbria fornix of hypoperfused SHR. Furthermore, terminal deoxynucleotidyl transferase-mediated dUTP in situ nick end labeling (TUNEL)-positive cells and lectin-positive microglia increased in number and intensities of staining more markedly in SHR than in WKY. In situ cell death detection ELISA supported these results quantitatively. RT-PCR represented the expression of TNF-[alpha], TNF receptor 1 (p55), caspase-2 (Ieh-1) and -3 (CPP32) mRNAs in both WKY and SHR brains after BCAL. Immunohistochemical analyses revealed that TNF-[alpha], TNF receptor 1 (p55), Ieh-1 and CPP32 immunoreactive cells could also be detected in the white matter regions of hypoperfused SHR. These results suggested that local production of TNF-[alpha] by the activated microglia might selectively induce oligodendroglial cell death through the death domain-containing TNF receptor 1 (p55), caspase-2 or -3 activation, resulting in white matter changes as a primary pathological feature.


http://www.sciencedirect.com/science/article/B6T0H-45WGH47-3/2/efc9d5d0fbb8da206bb1d1d914006ec3

Metallothionein (MT)-III is a metal binding protein, called growth inhibitory factor, and is mainly expressed in the central nervous system. Since MT-III decreases in the brain of Alzheimer's disease (AD), a growing interest has been focused on its relationship to neurodegenerative diseases. To clarify age-related changes in the MT-III expression and its inducibility against oxidative stress, we analyzed the expression of MT-III and its mRNA in the brain of lipopolysaccharide (LPS)-treated aged rats. In the frontal cortex, basal expression of MT-III mRNA was significantly increased with aging, while it was observed no induction of MT-III mRNA against LPS administration in the aged rat brain. MT-III immunopositive cells were increased in the frontal, parietal and piriform cortices, hypothalamus and amygdaloid nucleus with aging. The LPS treatment induced MT-III expression in the brain of young-adult rats, but not in the aged rat brain. Furthermore, the MT-III induction with LPS treatment was mainly observed in oligodendrocyte and microglia. In the present study, we showed that inducibility of brain MT-III against oxidative stress may be reduced with aging. Since it has been reported that MT-III has neuroprotective roles as an antioxidant, present results suggest that MT-III is closely related to the neurodegeneration in the aged animals.

To understand the basic mechanisms underlying neuronal differentiation, we have attempted to isolate differentially expressed genes, which may play a key role in this complex process, from neuronal differentiating P19 embryonal carcinoma cells. RNA fingerprinting by the arbitrarily primed PCR (RAP) method was adapted to detect such differentially expressed genes during P19 neuronal differentiation. Using this method with some modifications, we successfully cloned seven cDNA fragments which were expressed differentially within the first 48 h after 1 [mu]M retinoic acid (RA) treatment, which ultimately induces neuronal differentiation. Comparison of the partial nucleotide sequences of these clones with sequences in DNA databases indicated that one of these clones was identical to a region of the mouse Oct-3 gene, which has been shown to be dramatically repressed by RA. Two clones were highly homologous to the human profilinII and leucine-rich protein genes. The other four clones were not closely related to any sequences in the databases. Except for the Oct-3 gene, the other six genes isolated here have not been reported previously as RA-regulated genes. RAP is, thus, a promising method for identification of novel and potentially important genes which are differentially regulated during neuronal differentiation.


Membrane depolarization causes Ca2+ influx through L-type voltage-dependent calcium channels (L-VDCC), which promotes the activity-dependent survival of mouse cerebellar granule cells (CGCs). Although exogenously added pituitary adenylate cyclase activating polypeptide (PACAP) is effective in promoting the survival of CGCs, it is unknown whether PACAP is synthesized in CGCs and involved in the activity-dependent survival of CGCs. In this study, we found that the PACAP gene was activated in depolarized CGCs cultured at 25 mM KCl (high K+), independently of de novo protein synthesis. In addition, the PACAP immunoreactivity increased through the activation of L-VDCC in depolarized CGCs, indicating that PACAP is concomitantly produced with PACAP mRNA in an activity-dependent manner. Exogenously added PACAP attenuated the apoptosis of CGCs through a specific interaction with PACAP receptors. Furthermore, a PACAP receptor antagonist, PACAP(6-38), reduced the survival of CGCs at high K+. These findings indicate that endogenous PACAP production induced by Ca2+ signals exerts a survival effect on CGCs via PACAP receptors, which, at least in part, accounts for the activity-dependent survival of CGCs.


We have isolated cDNAs to two transcripts, granzyme M and alternative granzyme M mRNA from the mouse eye. Analysis of genomic DNA revealed these transcripts were derived from alternative transcription initiations. Northern blot analysis and reverse transcription-polymerase
chain reactions revealed that both transcripts were expressed in the eye, though the alternative form was the major type. In situ hybridization studies demonstrated that alternative granzyme M mRNA localized exclusively in the photoreceptor cells in the retina and expressed only after the opening of the eye, suggesting that these transcripts are related to the maintenance of the retinal structure or functions of matured photoreceptor cells rather than the development or differentiation of retinal cells.


http://www.sciencedirect.com/science/article/B6T0H-460WN7B-2/2/7899368d43f8cc1fad9f855f71adc25c

To determine the impact of hypertonic saline administration upon rat arginine vasopressin (AVP) gene transcription in supraoptic nucleus neurons, a probe complementary to the first intron (AVP1) of AVP was used to measure changes in AVP heteronuclear RNA (hnRNA) levels. Animals that received hypertonic saline had increases in AVP1 after 15 and 30 min, with a return to baseline levels by 180 min. In a double injection paradigm, animals were given an injection of normal or hypertonic saline followed 180 min later by a second injection of normal or hypertonic saline and sacrificed 30 min later. When both injections were hypertonic saline (H-H), AVP1 levels were greater than levels seen after a single hypertonic saline injection, or after an injection of normal saline followed by a second injection of hypertonic saline (N-H). This study shows acute, repeated exposure to hypertonic saline causes a robust increase in vasopressin gene transcription. Since a second hyperosmotic stimulus is known to increase neuronal firing rate and activity, our results suggest that a correlation exists with intracellular mechanisms regulating vasopressin gene transcription.

NeuroToxicology  (1)


http://www.sciencedirect.com/science/article/B6W81-436VY9B-5/2/a5310718b2f180b1ecac6b97089e7ef5

Exposure to high doses of the toxic organophosphate compound soman, also known as a chemical warfare agent, causes a progression of toxic symptoms including hyper-secrections, convulsions, respiratory depression, and finally death. In previous studies, we have demonstrated pronounced effects following soman intoxication in dopaminergic, GABAergic, and cholinergic systems in rat brain. The aim of this study was to investigate the effects on the pro-inflammatory cytokine interleukin-1[beta] (IL-1[beta]), indicated as mRNA and protein production, at different time intervals following soman intoxication. The peak levels of mRNA was observed 30 min following soman exposure, while a significant increase in the protein was observed at 6 h. Immunohistochemistry analysis revealed the presence of IL-1[beta] protein in astrocytes and endothelial cells. In addition to the previously observed effects of soman, there is an induction of IL-1[beta] in the brain. This effect, which is highly correlated to convulsions, implicates IL-1[beta] as a possible mediator for long-term brain damage observed after soman intoxication.

http://www.sciencedirect.com/science/article/B6WNT-4CBDC8M-1/2/ef492737c8a436451b82cca6449f9180

Echinococcus multilocularis and Echinococcus granulosus cause alveolar and cystic (unilocular) echinococcosis, respectively, in humans and animals. It is known that these parasites can affect, among other molecules, nitric oxide (NO) production by periparasitic host cells. Nevertheless, detailed dissection of parasite components specifically affecting cell NO production has not been done to date. We compare the effect of *E. granulosus* and *E. multilocularis* defined metacestode structural (laminated-layer associated) and metabolic (14-3-3 protein, potentially related with *E. multilocularis* metacestode tumor-like growth) components on the NO production by rat alveolar macrophages in vitro. Our results showed that none of these antigens could stimulate macrophage NO production in vitro. However, a reversed effect of some Echinococcus antigens on NO in vitro production was found when cells were previously exposed to LPS stimulation. This inhibitory effect was found when *E. multilocularis* laminated-layer (LL) or cyst wall (CW) soluble components from both species were used. Pre-stimulation of cells with LPS also resulted in a strong, dose-dependent reduction of NO and iNOS mRNA production after incubation of cells with the E14t protein. Thus, the *E. multilocularis* 14-3-3 protein appears to be one of the components accounting for the suppressive effect of the CW and LL metacestode extracts.


http://www.sciencedirect.com/science/article/B6WNT-49RCFTH-2/2/299fc4d5e9636de6128d0125971be938

Background. Nitric oxide (NO) is a free radical known to be a major regulator of vascular tonus, to inhibit cell proliferation, induce apoptosis, and be a mediator of macrophage cytostatic and cytotoxic effects. Recently, NO synthesis has been reported to be elevated in different cancers and is expected to promote metastasis by maintaining a vasodilator tone in blood vessels in and around the tumour. Two different common genetic polymorphisms were found on endothelial NO synthase (NOS3) gene: Glu298Asp on exon 7 and T->786C in the promoter region. Purpose. To evaluate the impact of the NOS3 polymorphisms on vascular invasion and metastasis in breast cancer patients. Design. Two NOS3 gene polymorphisms (Glu298Asp and T->786C) were genotyped in 71 patients operated for breast cancer and followed for 6-30 months (median 21). A control population of 91 age and sex matched tumour-free subjects was also genotyped for the same polymorphisms. Results. The distribution of both polymorphisms was not different between cases and controls. In patients without vascular invasion, T allele frequency was significantly lower than in patients with vascular invasion (p=0.033). At the end of the follow-up, T allele frequency was found to be less frequent in the metastasis free group than normal population (0.51 vs 0.64; p=0.047). Conclusion. Our results suggest that T allele reduction at the NOS3 promoter region may reduce vascular invasion in breast cancer and consequently reduce
metastatic spread and be a favorable prognostic factor. These results need further validation in larger studies.


http://www.sciencedirect.com/science/article/B6WNT-46XHFMS-3/2/a59898196c06b5704ea6f2cc614e3209

*Nuclear Medicine and Biology* (1)


http://www.sciencedirect.com/science/article/B6T9Y-4F1HS1N-C/2/19830bcec83b98622ad02b86e54e50ab

Methods have been developed to label oligonucleotides (ODNs) in the 5'-position with 76Br via a prosthetic group on a hexylamino-linker. The purpose of the study was to explore whether the labelling procedure would prevent specific hybridisation by using reverse transcription-polymerase chain reaction (RT-PCR) followed by sequencing of the PCR product. Antisense ODNs (30 mer, specific for rat Chromogranin A [CgA] mRNA) with phosphodiester (O-ODN) or phosphothioate (S-ODN) backbone, either unlabelled or labelled with 76Br, served as one of the primers in individual PCR reactions. Using O-ODN as a primer, irrespective of being labelled or not, a selected 225-bp PCR fragment was successfully amplified. However, no amplification was obtained using S-ODN as a primer. The proper PCR products were only detected in the sample prepared from the adrenal gland, but not in that from the heart, liver or kidney. Autoradiographic recording of the gel, after gel electrophoresis, revealed radioactive signals corresponding to the amplified PCR products. The sequence of the PCR product matched the rat CgA mRNA sequence obtained from the EMBL database. RT-PCR is an attractive method to identify the selective binding of modified ODNs to target mRNA. This method confirmed that the labelling with 76Br did not change the hybridisation ability of antisense O-ODN.

*Nutrition Research* (1)


http://www.sciencedirect.com/science/article/B6TB1-3YS2C5R-
We investigated whether amino acid substitution of tryptophan by arginine at the residue 64 (64 Arg) of beta 3-adrenergic receptor affects on the degree of reduction in the abdominal fat distribution during a 3-month weight reduction program in either pre- or postmenopausal Japanese women. Beta 3-adrenergic receptor gene polymorphism was examined in 90 Japanese obese women by restriction-enzyme cleavage conformation. The visceral and subcutaneous fat area was measured by magnetic resonance imaging. The baseline body mass index, body weight, fat mass and abdominal subcutaneous and total fat area in 15 obese postmenopausal women with a beta 3-adrenergic receptor (64 Arg) were significantly higher than those in 25 postmenopausal obese women with a beta 3-adrenergic receptor (64 Trp/64 Trp). In contrast, no such differences were found in the 50 premenopausal obese women. After a 3-month weight reduction period, the ratio of visceral to subcutaneous fat areas tended to be lower in both pre- and post-menopausal women with a beta 3-adrenergic receptor (64 Arg), but statistically significant in only the premenopausal obese women (p<0.05). The absolute changes in visceral fat areas in 5 homozygotes with a beta 3-adrenergic receptor (64 Arg/64 Arg) was significantly smaller than those in 50 obese women with a beta 3-adrenergic receptor (64 Trp/64 Trp). These results thus suggest that an amino acid substitution at residue 64 of beta 3-adrenergic receptor may play an important role in the regulation of fat distribution in Japanese obese women.

Obes. Res. (8)


http://www.obesityresearch.org/cgi/content/abstract/11/4/578

Objective: Elevated androgens in women are associated with type 2 diabetes and are dependent on the conversion to estrogens by aromatase cytochrome P450. Polymorphisms of a tetranucleotide repeat [TTTA]n in the fourth intron of the CYP19 gene are associated with endocrine-dependent diseases and were examined in relation to hormone levels and disease risk factors in premenopausal women. Research Methods and Procedures: A population sample of women born in 1956 (n = 270) were genotyped for this polymorphism and the results set in relation to steroid hormones, including saliva cortisol, anthropometric variables, estimates of insulin, glucose and lipid metabolism, and blood pressure. Results: Seven tetranucleotide repeat [TTTA]n alleles were detected with allelic sizes of 168 to 195 bp, with a TCT deletion/insertion (168/171 bp) upstream of this microsatellite. Smoking was associated with elevated androgens (p = 0.005 to 0.019). Using the median (average stretch, 177.5 bp) as a dividing line, nonsmoking women with the shorter microsatellite had higher free testosterone (p = 0.018) and lower sex hormone binding globulin (p = 0.033). These differences were pronounced with the 168-bp allele. Such women were also characterized by a less-substantial decrease of morning cortisols ("unwinding"; p = 0.035) and central obesity (abdominal sagittal diameter, p = 0.049) and had waist/hip circumference ratios of borderline significance (p = 0.064). Discussion: The results indicate that, in premenopausal women, a short microsatellite in the fourth intron of the CYP19 gene, caused by a TCT deletion upstream the [TTTA]n tract, is associated with elevated androgens, perturbed regulation of the hypothalamic-pituitary-adrenal axis, and abdominal obesity.

http://www.obesityresearch.org/cgi/content/abstract/11/7/809

Objective: To determine the contribution of the peroxisome proliferator-activated receptor {alpha} (PPAR{alpha}) L162V mutation to the variation of several indexes of body fatness obtained from healthy adults who participated in the Quebec Family Study. Research Methods and Procedures: The PPAR{alpha} L162V mutation was determined by a mismatch polymerase chain reaction method. Adiposity phenotypes were obtained by standardized anthropometric measurements, underwater weighing technique, and computed tomography. Results: For all adiposity phenotypes, subjects carrying the V162 allele had lower values compared with L162 homozygotes (HMZs) [BMI (kg/m2): 27.8 \(+/-\) 7.6 vs. 26.0 \(+/-\) 5.6, \(p < 0.05\); percentage body fat: 28.5 \(+/-\) 10.7 vs. 25.7 \(+/-\) 10.1, \(p < 0.05\); waist circumference (cm): 89.0 \(+/-\) 18.1 vs. 85.7 \(+/-\) 15.8, \(p = 0.07\); total computed tomography abdominal fat areas (cm2): 406 \(+/-\) 221 vs. 359 \(+/-\) 192, \(p = 0.15\); means \(+/-\) SD for L162 HMZs vs. V162 carriers, respectively]. Differences in cross-sectional abdominal adipose tissue areas and waist circumference were abolished after adjustment for total body fat mass. Similar trends were observed when results were analyzed by gender, although associations seemed stronger in women. The odds ratio of having a BMI above 30 kg/m2 reached 1.77 (1.02; 3.07, 95% confidence intervals) for L162 HMZs. This risk could be considered marginal on an individual basis, but because 85% of the subjects are affected by this small risk, the impact on the population is important. Discussion: The PPAR{alpha} V162 allele is associated with reduced adiposity and has a substantial population-attributable risk.


http://www.obesityresearch.org/cgi/content/abstract/12/4/734

Objectives: To verify whether Infliximab could modify insulin sensitivity and TNF-(alpha) and GLUT4 mRNA expression in muscle and adipose tissue of morbidly obese subjects. Soluble TNF receptors I and II (TNFR-I and TNFR-II) were also assayed. Research Methods and Procedures: Six obese subjects were investigated before and 2 weeks after a single intravenous administration of 5 mg/kg Infliximab; insulin sensitivity was evaluated by euglycemic hyperinsulinemic clamp, and TNF-(alpha) and GLUT4 mRNA expression were assessed by reverse-transcriptase polymerase chain reaction on muscle and adipose tissue. TNF-(alpha), TNFR-I, and TNFR-II were determined using the ELISA technique. Results: Infliximab infusion did not affect fasting plasma insulin or fasting plasma glucose levels; whole body glucose uptake did not change significantly. TNF-(alpha) and GLUT4 mRNA did not show any significant change in muscle or adipose tissue. Serum TNF-(alpha) was undetectable before and after treatment, whereas TNFR-I and TNFR-II concentrations significantly decreased (\(p < 0.01\)). Discussion: An explanation for the absence of effect of Infliximab on insulin resistance in morbidly obese subjects may be the paracrine way of action of this cytokine. Because Infliximab is predominantly distributed within the vascular compartment, its effectiveness in penetrating muscle and adipose tissue is potentially low. The significant decrease of TNFR-I and TNFR-II might be ascribed to a targeted effect of Infliximab on the immune system.


http://www.obesityresearch.org/cgi/content/abstract/13/3/519
We assessed interactions between polymorphisms in the \(\beta\)-adrenergic receptor genes and longitudinal changes in obesity from childhood to adulthood using longitudinal data collected over a 24-year period from 1973 to 1996. Sex- and age-stratified analyses using random coefficients models were used to examine gene-gene interaction effects on obesity measures in 1179 African-American and white men and women (71% white, 57% women). Suggestive evidence for an interaction (p = 0.022) between the \(\beta_1\) and \(\beta_2\)-adrenergic receptors was observed in men for longitudinal change in BMI. Men with Gly/Gly genotypes for both the \(\beta_1\) and \(\beta_2\) receptors showed significant increases ([~]0.6%/yr) in BMI from childhood to adulthood. Women showed suggestive evidence for an interaction (p = 0.035) between the \(\beta_1\)- and \(\beta_3\)-adrenergic receptors for change over time in BMI. Women with Gly/Gly genotypes at the \(\beta_1\)-receptor and carrying at least one \(\beta_3\)-Arg allele showed notable increases in BMI. The regulation of lipolysis and development of obesity differ markedly between men and women and may be influenced by genetic polymorphisms, which contribute to the efficiency of the \(\beta\)-adrenergic receptors, and hormonal effects on adrenergic receptor activity.


http://www.obesityresearch.org/cgi/content/abstract/10/12/1232

Objective: To assess the association between a polymorphism related to dopamine function, dopamine transport (SLC6A3), and obesity in smokers. Research Methods and Procedures: Logistic regression was used to assess the relationship between this genetic polymorphism and obesity (body mass index \(\geq 30\) kg/m\(^2\)) from a sample of 510 smokers who smoked at least 10 cigarettes per day and who were participating in a study designed to examine genetic and nongenetic predictors of response to a pharmacological treatment. Results: The likelihood of obesity in African Americans (N = 90) with the 10/10 SLC6A3 genotype was 5.16 times that of African Americans with 9/9 or 9/10 SLC6A3 genotypes (odds ratio = 5.16, confidence interval = 1.60 to 16.65). There was no association of the SLC6A3 genotype with obesity for non-Hispanic whites (N = 420). Discussion: These results suggest that variants of the dopamine transporter gene may be related to obesity in African-American smokers. Possible mechanisms responsible for the association between dopamine transport and obesity in African-American smokers are discussed.


http://www.obesityresearch.org/cgi/content/abstract/13/3/574

Objective: Alternate day fasting may extend lifespan in rodents and is feasible for short periods in nonobese humans. The aim of this study was to examine the effects of 3 weeks of alternate day fasting on glucose tolerance and skeletal muscle expression of genes involved in fatty acid transport/oxidation, mitochondrial biogenesis, and stress response. Research Methods and Procedures: Glucose and insulin responses to a standard meal were tested in nonobese subjects (eight men and eight women; BMI, 20 to 30 kg/m\(^2\)) at baseline and after 22 days of alternate day fasting (36 hour fast). Muscle biopsies were obtained from a subset of subjects (n = 11) at baseline and on day 21 (12-hour fast). Results: Glucose response to a meal was slightly impaired in women after 3 weeks of treatment (p < 0.01), but insulin response was unchanged. However, men had no change in glucose response and a significant reduction in insulin response (p < 0.03). There were no significant changes in the expression of genes involved in mitochondrial biogenesis or fatty acid transport/oxidation, although a trend toward increased CPT1 expression was observed (p < 0.08). SIRT1 mRNA expression was increased after alternate day fasting (p =
Discussion: Alternate day fasting may adversely affect glucose tolerance in nonobese women but not in nonobese men. The gene expression results indicate that fatty acid oxidation and mitochondrial biogenesis are unaffected by alternate day fasting. However, the increased expression in SIRT1 suggests that alternate day fasting may improve stress resistance, a commonly observed feature of calorie-restricted rodents.


http://www.obesityresearch.org/cgi/content/abstract/10/9/932

Objective: Genetically obese (fa/fa) Zucker rats display markedly elevated circulating leptin levels compared with their lean counterparts; this is expected because of the lack of a LepR-mediated feedback inhibition. The aim of this study was to determine the effect of the leptin receptor mutation in the Zucker rat on gastric leptin production and on the response to 14 hours of starvation. The response to a short-term period of food intake (20 minutes) on gastric leptin release was also analyzed. Research Methods and Procedures: Leptin mRNA expression in the gastric mucosa and in adipose tissue depots (epididymal, retroperitoneal, mesenteric, and inguinal) was assessed by reverse transcriptase-polymerase chain reaction and serum and stomach leptin content by enzyme-linked immunosorbent assay. Results: Obese Zucker rats overexpressed leptin in the stomach. They overexpress leptin in the inguinal adipose tissue but not in visceral adipose tissue depots, indicating tissue-specific obesity-dependent differences. Gastric leptin expression is regulated by feeding conditions in lean but not in obese (fa/fa) rats. In lean animals, leptin mRNA levels decrease in fasting conditions and increase rapidly with a short period of food intake. Obese Zucker rats also overdisplay stomach leptin levels. Feeding acutely stimulates leptin secretion by the stomach in lean, and to a lesser extent, in obese rats. Discussion: These results indicate impaired regulation of leptin expression in the stomach of obese (fa/fa) Zucker rats. However, there is still an effect of the nutritional status on gastric leptin levels despite the lack of a functional leptin receptor.


http://www.obesityresearch.org/cgi/content/abstract/11/2/176

Objective: The aim of this study was to verify whether changes in PDK4 mRNA expression in skeletal muscle in formerly obese subjects who underwent malabsorptive bariatric surgery [bilio-pancreatic diversion (BPD)] might be related to insulin sensitivity improvement, and if these possible modifications might correlate with a reduction of the intramyocytic lipid level. Research Methods and Procedures: Six obese women (body mass index 46.6 {+/-} 8.2 kg/m2) were enrolled in the study. Body composition, euglycemic-hyperinsulinemic clamp and muscle biopsies for skeletal muscle lipid analysis, and semiquantitative reverse transcriptase-polymerase chain reaction were performed before and 3 years after BPD. Results: The average weight loss observed after surgery was [~]42%. Increased glucose uptake was accompanied by a significant decrease of PDK4 mRNA (R2 = 0.71, p < 0.001). The amounts of intramyocytic triglycerides correlate directly with PDK4 mRNA (R2 = 0.87, p = 0.005) and inversely with glucose uptake values (R2 = 0.75, p < 0.001). Discussion: Our results support the concept that a reduced tissue availability of fatty acids consequent to a massive lipid malabsorption influences glucose metabolism acting through the regulation of PDH complex. In fact, as shown in animals, a higher level of FFA availability is likely to induce overexpression of PDK4 also in humans.
OBJECTIVE: To examine the concordance between self-collected and clinician-collected samples for human papillomavirus (HPV) DNA. METHODS: Sexually active adolescent and young adult women aged 14-21 years (N = 101) were enrolled in a prospective cohort study of HPV testing. Participants self-collected vaginal samples for HPV DNA, and clinicians collected cervicovaginal samples for HPV DNA and a cervical cytology specimen. We determined concordance between the results of self- and clinician-collected specimens using a \( \kappa \) statistic and McNemar's test. RESULTS: Of the 51% of participants who were HPV positive, 53% had 1 type, 25% had 2 types, and 22% had 3 types or more; 25 different HPV types were identified. Self-collected samples detected more participants with HPV than clinician-collected samples (45% versus 42%, \( P = .65 \)). When results were categorized into presence or absence of high-risk HPV types, agreement between self- and clinician-collected specimens was high \((\kappa) 0.72\) and the difference between test results was not significant (McNemar's \( P = .41 \)). However, when all HPV types detected were considered, agreement was perfect in only 51% of those with 1 or more types of high-risk HPV type. There was no association between agreement and age or HPV type. CONCLUSION: Self testing for HPV DNA may be sufficiently sensitive for the detection of high-risk HPV DNA among adolescent and young adult women in clinical settings. LEVEL OF EVIDENCE: II-3
Conclusion: The infection rate of 24.6% in women without clinical symptoms of HPV infection was high, but there seemed to be no virus transmission to the placenta in women with subclinical infections. Low-risk cervical HPV infection might be associated with a slightly higher risk of abnormal fetal karyotype.


http://www.sciencedirect.com/science/article/B6TB2-496F1R7-D/2/eddeabcaf772d718487ffa51dbbff6b

ObjectiveTo compare the performance of patient- and physician-obtained cytology and human papillomavirus (HPV) testing for the detection of high-grade cervical intraepithelial neoplasia.

MethodsA cross-sectional study was performed involving 334 women seen at three colposcopy clinics (Tucson, Arizona; Hermosillo, Mexico; and Lima, Peru). All women used a cytobrush to self-collect specimens for cervical cytology and HPV testing. Subjects subsequently underwent physician collection for cytology and HPV, followed by a complete colposcopic evaluation with directed biopsy. Cytology was processed using thin-layer technology, and HPV was determined using the polymerase chain reaction technique. Test performance characteristics were determined using the histopathologic diagnosis as the reference standard and designating high-grade cervical intraepithelial neoplasia as clinically significant disease for the purpose of the analysis.

ResultsThe sensitivity of patient-collected cytology was significantly lower (55.0%) and specificity was significantly better (84.1%) than those of physician-directed sampling (85.2% and 73.4%, respectively). Patient-collected HPV had significantly lower sensitivity (49.0%) than physician sampling (82.2%), although specificity did not significantly differ.

ConclusionPatient collection is a feasible although inferior alternative to physician-collected cervical cytology and HPV testing.


http://www.sciencedirect.com/science/article/B6TB2-4B4P70J-V/2/3bc6092821c4002928b97e54462d7c72

ObjectiveTo study the association between serum human papillomavirus (HPV) deoxyribonucleic acid (DNA) and clinicopathologic prognostic factors and the clinical usefulness of serum HPV DNA in early-stage cervical cancer.

MethodsDeoxyribonucleic acids extracted from cervical tissues and sera of patients with stage IB or IIA cervical cancer and 40 controls including patients with cervical carcinoma in situ or benign disease were examined for HPV DNA with L1 consensus and types 16- and 18-specific E7 primers. Multivariable logistic regression was used to determine significant correlates of positive serum HPV DNA, and the receiver operating characteristic curve was applied in risk-factor assessment.

ResultsHuman papillomavirus DNA was not detected in sera from patients with carcinoma in situ or benign disease. Among the 112 patients with cervical cancer, we detected 27 positive samples (24.1%) in serum. Positive HPV DNA in serum was significantly associated with lymphovascular invasion and deep stromal invasion with or without parametrial extension (P = .001), large tumor size, and elevated levels of serum squamous cell carcinoma antigen (P = .001). The presence of serum HPV DNA in patients with early-stage cervical cancer was correlated with poor prognosis factors that warrant adjuvant therapy.

http://www.sciencedirect.com/science/article/B6TB2-40SFFDM-T/2/4be1f52155ddca619cd66623ab40aa24

Objective: To evaluate C to T substitution at nucleotide 677 of N5,N10-methylenetetrahydrofolate reductase gene in women with prior preeclamptic or normotensive pregnancies.

Methods: Methylenetetrahydrofolate reductase genotypes were determined in 113 Finnish women with preeclamptic first pregnancies and 103 controls with one or more normotensive pregnancies, using polymerase chain reaction and restriction enzyme analysis. Preeclampsia was defined as severe in 100 women who fulfilled one or more of the subsequent criteria: systolic blood pressure (BP) at least 160 mmHg, diastolic BP at least 110 mmHg, or proteinuria at least 2 g per 24-hour urine collection.

Results: There were no significant differences in prevalences of the methylenetetrahydrofolate reductase genotypes (CC, CT, and TT) between groups (57%, 40%, and 3% in the preeclamptic group and 54%, 39%, and 7%, respectively, in controls). The frequency of the T677 allele was 0.23 in the preeclamptic group and 0.26 in the control group (difference 0.03; 95% confidence interval -0.08, 0.14; P =.51). Our sample had 60% power to detect a difference of the allele frequencies similar to that (0.12) reported previously. The result was similar when analysis was restricted to patients with severe preeclampsia (T677 allele frequency 0.22). Conclusion: A carrier status for the T677 allele of the methylenetetrahydrofolate reductase gene does not predispose to preeclampsia, at least in the Finnish population.


http://www.sciencedirect.com/science/article/B6TB2-3Y6GTY5-W/2/bda8415a152ed169e7ca64c01499ce0d

Objective: The role of human leukocyte antigen (HLA) DQB1 alleles and human papillomavirus (HPV) as contributing factors to invasive cervical cancer was investigated. To overcome problems of misleading causal inferences common in traditional case-control studies, a family-based test, the transmission/disequilibrium test, was used.

Methods: Ninety-six patients with pathologically confirmed invasive cervical cancer were ascertained. Human papillomavirus types were determined in 80 patients, of whom 81.25% were HPV-positive, and 18.75% were HPV-negative. Deoxyribonucleic acid was extracted from samples, taken from patients and their parents, and sequenced to determine DQB1 genotypes. Nuclear family data were used to test whether the DQB1 locus is associated with invasive cervical cancer while controlling for high-risk HPV-positive patients. The transmission/disequilibrium test evaluates whether the frequency of transmission of parental marker alleles to their affected offspring deviates from the expected Mendelian frequency of 50%.

Results: The HLA DBQ1 locus showed evidence for allelic association with invasive cervical cancer in high-risk HPV-positive patients (P = .006). The transmission/disequilibrium test showed that the DQB1*0303 allele was transmitted to high-risk HPV patients more often than expected by chance, [chi]21 = 8.0, P = .005 (P = .035 when correcting for multiple tests). Tests of association were negative when applied to all 96 patients, irrespective of HPV status. No significant differences were found in the distribution of the DQB1 alleles among HPV-positive patients compared with those who were HPV-negative, indicating that HLA alleles are not associated with susceptibility to HPV infection.

Conclusion: These results suggest that the DQB1*0303 allele increases the risk for invasive cervical cancer in women who are HPV-positive.

Objective: To examine whether concentrations of free extracellular fetal circulatory DNA in maternal plasma are stable or fluctuate.

Methods: Consecutive blood samples were drawn from 13 healthy nonpregnant volunteers and from 16 healthy pregnant women over 3 days. DNA was isolated from the plasma fraction and quantified by real-time polymerase chain reaction (PCR).

Results: In nonpregnant controls the total amount of cell free DNA fluctuated by an average of 13.5-fold. In samples obtained from pregnant women the amount of maternal cell free DNA varied by an average of 21.5-fold. Because ten of those women were pregnant with male fetuses, the concentration of free fetal DNA in these cases was determined by a real-time PCR assay for the Y chromosome. The mean variation in free fetal DNA levels in male fetuses was 2.2-fold.

Conclusion: The degree of variation in free fetal DNA concentrations observed in this study was similar to published values, so these results imply that care should be exercised when considering quantitation of this fetal material for potential diagnostic or screening purposes.


ObjectiveTo investigate the role of herpes viruses in the etiology of serpiginous choroiditis.

DesignInterventional case report.

ParticipantA 59-year-old male patient with long-term history of serpiginous choroiditis.

InterventionThe patient's affected eye was obtained during autopsy. Polymerase chain reaction was performed in the microdissected choroidal tissues.

ResultsHistopathologic examination demonstrated active inflammation with lymphocytic infiltration of the choroid. No viral DNA was amplified using pairs of herpes simplex virus (HSV) P1/P2 (for HSV-1, HSV-2, Epstein-Barr virus [EBV], cytomegalovirus [CMV] and human herpes virus [HHV]-8), and varicella-zoster virus [VZV] P1/P2 (for VZV, HHV-6, HHV-7) in the infiltrating lymphocytes or choroidal tissues.

ConclusionsThe current observation suggests a lack of a role for herpetic viral etiology in the etiopathogenesis of serpiginous choroiditis.


ObjectiveTo show the use of the polymerase chain reaction (PCR) in a granulomatous choroidal lesion to support a diagnosis of tuberculosis.

DesignObservational case report.

TestingNucleic acid target amplification of a choroidal specimen using PCR for detection of Mycobacterium tuberculosis was tested.

Main outcome measuresPositive nucleic acid target amplification for M.
tuberculosis in the ocular sample was measured. Results PCR was positive for M. tuberculosis with appropriate negative controls. Conclusions PCR was thought to be a useful supportive technique in the diagnosis of choroidal tuberculosis.


http://www.sciencedirect.com/science/article/B6VT2-41HHMCS-1D/2/5ec0f5b61f1bb75606d8107bcb15495b

Objective Primary graft failure (PGF) corneal tissues were analyzed for herpes simplex virus (HSV) and varicella-zoster virus (VZV). Design Retrospective, noncomparative case series. Materials Formalin-fixed, paraffin-embedded tissue of 21 donor corneas and 14 recipient corneas of PGF cases, as well as 10 control corneas. Methods Clinical, histologic, immunohistochemical, polymerase chain reaction (PCR), and, in selected cases, transmission electron microscopic characteristics were studied. Main outcome measures Evidence of HSV or VZV in donor tissues. Results Median patient age was 65 years, and median donor age was 48 years. Donor cornea parameters, including endothelial cell counts, death-to-preservation time, and time in storage, were generally within accepted standards. Stromal edema was found in all 21 donor corneas with PGF. Eighteen donor corneas demonstrated severely reduced or absent endothelium and mild to moderate lymphocytic infiltration without necrosis. Three donor corneas (14%) had necrotizing stromal keratitis (NSK) with keratic precipitates. Positive immunohistochemical staining of keratocytes for HSV was present in two of two donor corneas with NSK and was negative in 18 other donor corneas. Polymerase chain reaction analysis revealed the DNA of HSV type 1 (HSV1) in all donor corneas with NSK and in four donor corneas without NSK (33%). Recipient corneal tissue was negative for HSV1 DNA in three patients with NSK and positive in two of the four other PCR-positive patients. Transmission electron microscopy analysis showed viral particles in two donor corneas with NSK. Polymerase chain reaction analysis revealed no evidence of HSV type 2 or VZV in any cornea. All control corneas were negative for viral DNA. Sixteen corneas remained clear and two had failed after regraft for PGF, with a median follow-up of 3.6 years. Conclusions Herpes simplex virus type 1 DNA was present in 33% of patients of PGF. Herpetic stromal keratitis was found in some failed corneas; the lack of HSV in the paired recipient suggests importation within the donor cornea. The overall prognosis for regrafting after PGF is good.


http://www.sciencedirect.com/science/article/B6VT2-49CMCY2RD/2/602ccaf9b43c1d701978c94ce4dd734f

Objective Lymphoproliferative lesions of the ocular adnexa were analyzed to examine (1) the suitability of the Revised European-American Lymphoma (REAL) classification for the subtyping of the lymphomas in these sites; (2) the predictive value of the REAL classification for the evolution of these tumors; and (3) the frequency and prognostic impact of tumor type, location, proliferation rate (Ki-67 index), p53, and CD5 positivity and the presence of monoclonality within these tumors. Design Retrospective review. Methods The clinical, histomorphologic, immunohistochemical, and molecular biologic (polymerase chain reaction [PCR]) features of lymphoid proliferations of the ocular adnexa were studied. Study materials The ocular adnexal lymphoproliferative lesions were located as follows: orbit in 52 patients (46%), conjunctiva in 32 patients (29%), eyelid in 23 patients (21%), and caruncle in 5 patients (4%). Results Reactive lymphoid hyperplasia was diagnosed in 12 cases and lymphoma in 99 cases; 1 case remained
indeterminate. The five main subtypes of lymphoma according to the REAL classification were extranodal marginal-zone B-cell lymphoma (64%), follicle center lymphoma (10%), diffuse large cell B-cell lymphoma (9%), plasmacytoma (6%), and lymphoplasmocytic lymphoma (5%). Age, gender, and anatomic localization of the lymphomas did not have prognostic significance during a follow-up period of 6 months to 16.5 years (mean, 3.3 years). Extent of disease at time of presentation was the most important clinical prognostic factor: advanced disease correlated with increased risk ratios of having persistent disease at the final follow-up and with lymphoma-related death (P P P P P ConclusionThe REAL classification is suitable for the subdivision of the ocular adnexal lymphomas. The MIB-1 proliferation rate and p53 positivity may aid the prediction of disease stage and disease progression, whereas PCR can support the diagnosis and reduce the number of histologically indeterminate lesions.


http://www.sciencedirect.com/science/article/B6VT2-49CR821-17/2/6c52a0477b7d2a1c4d41405f5cbba0b6

BackgroundSebaceous carcinoma may masquerade for years as an inflammatory condition. In many cases, this may be because of the presence of longstanding intraepithelial disease (e.g., dysplasia or carcinoma in situ), which eventually progresses to invasive carcinoma recognized through tumefaction and a worsening clinical presentation. The mechanism for this tumor progression is unknown. In the Far East, human papilloma virus (HPV) has been suggested to play a role in the development of sebaceous carcinoma by inactivating tumor suppressor gene p53. Here, the authors explore the molecular basis of the progression of ocular sebaceous carcinoma.MethodsCases of sebaceous carcinoma seen at the University of Virginia, Department of Ophthalmology, during the period from 1989 to 1996 were analyzed for HPV infection by in situ hybridization and polymerase chain reaction. The expression of p53, p21WAF-1, Bcl-2, and epithelial membrane antigen was examined by immunohistochemistry. In one of the cases, frozen tumor was available, allowing exons 5 through 9 of the p53 gene to be sequenced.ResultsSeven cases were identified, all of which were from women. All were negative for HPV. In cases in which disease was restricted to dysplasia (carcinoma in situ), p53 but not p21WAF-1 was negative. In contrast, cases that contained a component of invasive or metastatic carcinoma showed striking hyperexpression of nuclear p53 in all of the malignant cells. In one of these cases, a G:C -> T:A transversion was found in the p53 gene. This mutation, characteristic of bulky carcinogens, substituted phenylalanine for cysteine 277, a residue that participates in hydrogen bonding to the p53 DNA binding consensus sequence.ConclusionsMutational inactivation of p53 may be involved in the progression of sebaceous carcinoma.

Oral Oncology (14)


http://www.sciencedirect.com/science/article/B6TB6-3Y51HW4-K/2/e14d7e80469b4efec80a6d68ad652a6ed
A recently identified putative tumor-suppressor gene, PTEN, at 10q23 has been described as mutated or homozygously deleted in many different human tumors. To determine the role of the homozygous deletion of this PTEN gene in oral squamous cell carcinoma (OSCC), we screened two cell lines derived from the latter tissue and 28 tumor samples from patients with OSCC, using a differential display polymerase chain reaction (PCR) system and, direct DNA sequencing methods. All of the nine exons of the PTEN could be successfully amplified using DNA from tumor tissues and the cell lines using this system. DNA sequencing confirmed the accuracy of the PCR procedures. However, none of the samples, either from the cancer tissues or from the cell lines, showed homozygous deletion of PTEN. These data suggest that homozygous deletion of the PTEN gene is unlikely to be a feature of OSCCs.


http://www.sciencedirect.com/science/article/B6TB6-3Y2MYYO-1C/2/44fcd149be38a4d856d765a9c97a17d8

The aetiology of oral cancer is thought to be multifactorial. Apart from the two known major risk factors (tobacco and alcohol), a viral aetiology has been proposed, with special reference to human papillomavirus (HPV). 35 cases of oral squamous cell carcinoma (OSCC), seen at the Departments of Oral & Maxillofacial Surgery and Oral Pathology and Otologyngology of the Free University of Amsterdam, were analysed as well as 12 biopsies of clinically and histologically normal gingival mucosa collected from healthy individuals after tooth extractions, using the polymerase chain reaction (PCR) and two different sets of primers that are able to detect a broad spectrum of HPV types. An overall HPV positivity of 54.3% in OSCC was found, the majority of positive cases (78.9%) harbouring HPV type 16. In contrast, no positivity for HPV was detected in the clinically normal oral mucosal samples analysed. Furthermore, a significant association between HPV presence and age was found: patients older than 60 years showed a lower prevalence of the virus (29.4%) compared with patients below this age (77.8%) (P < 0.05). The results from the present study suggest an association between HPV and OSCC, particularly in patients under the seventh decade.


http://www.sciencedirect.com/science/article/B6TB6-3VYXY78-K/2/d3c9364fe47cb02207fff69e3b9a2f01

The presence of high risk human papilloma virus (HPV) 16 and 18 was examined in 100 oral cancer patients of Indian descent, 80 patients with potentially malignant oral lesions and corresponding clinically normal mucosa from 48 of these patients. Additionally, presence of HPV-33, -6 and -11 was also studied in 86 oral cancers, 50 potentially malignant oral lesions and 30 corresponding normal oral mucosa. All the patients with oral cancer and oral lesions, were long term tobacco-chewers, and a majority of the patients were in Advanced Stages III and IV. The DNA samples were amplified by polymerase chain reaction (PCR) using HPV L1 consensus primers. Typing of HPV was performed by Southern hybridization analysis of the PCR products using HPV-16, -18, -33, -6 and -11 type specific oligonucleotide probes. HPV-16 was detected in 15 out of 100 (15%) oral tumours, 27 out of 80 (34%) potentially malignant lesions and 15 out of 48 (31%) of the corresponding normal mucosa in the patients with oral lesions. HPV-18 was not detected in any of the oral cancers, oral lesions and normal mucosa. HPV-33 and the low-risk HPV-6 and -11 were also not detected in the oral cancers, oral lesions and corresponding normal mucosa. A significantly higher prevalence of HPV-16 was observed in oral lesions (27 out of 80,
The observed difference of 19% (95% confidence interval [CI]: 6%, 31%), between these two proportions was statistically significant at the 5% level of significance. Our data indicates that HPV-16 may play a direct role in a certain proportion of oral cancers; whereas in a subpopulation of oral cancers HPV-16 infection may be vital in the early events associated with development of potentially malignant oral lesions, and the presence of the virus not essential in the progression of the oral lesion to frank malignancy.


http://www.sciencedirect.com/science/article/B6TB6-49BY4C9-9/2/141879f1015466e1b8a62fe26f7b0911

Tumors show an increased glucose uptake that is mediated by glucose transport proteins. We have analyzed the expression of the sodium-dependent glucose co-transporters SGLT-1 and-2 in short-term cultures of squamous cell carcinomas of the head and neck (HNSCC) by RT-PCR. Distribution of the SGLT-1 protein in HNSCC tissues was investigated by immunohistochemistry. While we observed in 17/36 HNSCC short-term cultures the SGLT-1 mRNA, we found no SGLT-2 expression. SGLT-1 mRNA expression occurred preferentially in cultures originating from moderately and well differentiated HNSCC. In tumor tissues a heterogeneous SGLT-1 staining restricted to differentiated tumor cells was seen in 27/30 HNSCC analyzed. In normal mucosa SGLT-1 staining was also confined to differentiated compartments and lacked in dysplastic areas. Our data indicate a differentiation-dependent expression of SGLT-1 in HNSCC. This is important knowledge for the planning of glucose-targeting therapies and suggest SGLT-1 as a differentiation marker in head and neck tissues.


http://www.sciencedirect.com/science/article/B6TB6-460WNHM-1/2/d0534d71c2f4ffcc2ab6bb20906bf004

Expression profile of 588 known genes relating to tumour biology, was examined between oral squamous cell carcinomas (OSCCs) and matching normal oral mucosal tissues (NOMTs) obtained from Sudanese (n=11) and Norwegian (n=11) patients. cDNA probes were synthesised from total RNA and hybridised with the Atlas human cancer cDNA expression array membranes. RT-PCR and immunohistochemistry were applied to confirm the expression pattern of a subset of the 588 genes. Differences in expression of the genes examined were found between the OSCCs and the NOMTs on the Atlas membranes. Several of these genes were either up- or down-regulated 1.6-fold or higher in the OSCCs compared to the NOMTs in the cases from the two populations. We found that 181 (31%) and 195 (33%) genes were either up-regulated or down-regulated in the OSCCs from the Sudan and Norway, respectively. From the total number of genes (n=376) found expressed in the OSCCs investigated from the two countries, 53 genes (14%) showed common expression profile [35 (66%) were up-regulated and 18 (34%) were down-regulated] and 70 genes (19%) showed opposite regulation status. Results of the RT-PCR and immunohistochemistry confirmed the hybridisation data. These findings may provide an OSCCs-specific gene expression profile in patients from the two countries, suggesting that alterations of 123 genes are common in these OSCCs regardless of ethnic differences or other socio-cultural risk factors between the patients from the two countries. The findings might further suggest that specific genes are frequently involved in these OSCCs, which may provide novel clues as diagnostic, prognostic biomarkers and/or targets for therapy. The Atlas human cancer
cDNA expression array technique can be useful to examine and describe the expression profile of known genes frequently involved in OSCCs from different populations.


http://www.sciencedirect.com/science/article/B6TB6-3W19WVP-D/2/62eca3ee658bdecea90437db6beb0015

Using immunohistochemistry, expression of p53, transforming growth factor-alpha (TGF-[alpha]), epidermal growth factor receptor (EGFR), c-erbB-2/neu and proliferating cell nuclear antigen (PCNA) was examined in 26 fresh frozen tissue specimens of oropharyngeal squamous cell carcinomas (SCCs). p53 gene mutations were examined by polymerase chain reaction (PCR)/DNA sequencing methods in 22 carcinomas. The findings were examined for correlations with patients' clinicopathological parameters. Expressions of p53 and PCNA were also examined in 21 formalin-fixed corresponding tissues. Of the fresh frozen tissue specimens, 77% (20/26) showed expression and 68% (15/22) showed mutations (substitutions) of the p53, with significant clustering of the mutations in exons 5 (8/22; 36%), 7 (4/22; 18%) and 8 (5/22; 23%). No mutations were found in exon 6. There was a discordance between expression of p53 protein and mutations of the gene. Parallel to expression and mutations of the p53 found in most of the specimens, expression of TGF-[alpha], EGFR, c-erbB-2/neu and PCNA was found in 88% (22/25), 92% (23/25), 58% (14/24) and 91% (21/23) of the specimens, respectively. For the formalin-fixed tissue specimens, 62% (13/21) and 90% (19/21) expressed p53 and PCNA, respectively. Examining for correlations with patients' clinicopathological parameters, expression of p53, TGF-[alpha], EGFR and c-erbB-2/neu seemed to negatively correlate with the increase of the tumour grade. The present work suggests that: (1) lack of negative growth regulation due to inactivation of the p53 gene together with activation of other proto-oncogenes are necessary genetic events in the carcinogenesis of oropharyngeal SCCs; (2) in oropharyngeal SCCs, p53 gene mutations were clustered in exons 5 (codons 130-186), 7 (codons 230-248) and 8 (codons 271-282) which perhaps suggests that tobacco carcinogens probably affect the mutational hot spots of the p53 gene at codons 157, 175, 186, 248, 273 and 282; and (3) fresh frozen and formalin-fixed tissue specimens give similar results when an immunohistochemical method is applied. The importance of p53, TGF-[alpha], EGFR, c-erbB-2/neu and PCNA as biomarkers in oropharyngeal SCCs deserves particular attention because it might offer further understanding of the development of these carcinomas.


http://www.sciencedirect.com/science/article/B6TB6-43YR0B8-5/2/3ef78ec1804098fb87eb59e301008c09

Overexpression and amplification of several genes (MDM2, CDK4 and SAS) located on chromosome 12q13-15 have been noted to occur in various human sarcomas. As a result, two major growth regulation pathways may be inhibited. MDM2 may down regulate the p53-mediated growth control and CDK4 may affect pRB-mediated events. To determine the frequency of alterations in these genes and their correlation with clinicopathologic features, we analyzed the MDM2 and CDK4 protein levels by immunohistochemistry and assessed MDM2, CDK4 and SAS amplification by real-time PCR in nine osteosarcomas of the jaws. Positive staining for CDK4 and MDM2 was observed in eight cases (88.8%) and five cases (55.5%), respectively. Intense CDK4 staining was noted in four cases (two high grade, one intermediate grade and one low grade).
Intense MDM2 staining was observed in the same four previous cases, as well as, one additional high-grade tumor. Individual DNA amplification for CDK4, MDM2 and SAS was observed in six cases for each gene. Co-amplification was observed in five cases that showed CDK4 and MDM2 concomitant amplification and four cases that displayed amplification for all of the genes. In addition, among the five cases that presented CDK4 and MDM2 amplification, strong overexpression of CDK4 and MDM2 was observed in three and in four cases, respectively (three high grade and one intermediate grade). These results suggest that 12q13-15 genes are involved in neoplastic disease and concurrent amplification and overexpression of these genes might help to define high-grade tumors.


Although an important risk factor for oral cancer is the presence of epithelial dysplasia, most of these lesions will not progress to malignancy. Presently, for the individual patient with dysplasia, there are few reliable markers that may indicate the likelihood of progression to oral cancer. Cathepsin L is a lysosomal protease that degrades extracellular matrix material. Because cathepsin L is frequently overexpressed in oral squamous cell carcinoma (SCC) we hypothesized that it is also overexpressed in oral premalignancy and that premalignant lesions that progressed to oral cancer expressed higher levels of cathepsin L than those premalignant lesions that did not. In this retrospective pilot study we examined changes in cathepsin L expression at the mRNA level using quantitative TaqMan RT-PCR and at the protein level by immunohistochemistry in 33 routinely processed oral dysplastic lesions and 14 SCCs obtained from 33 patients. Sixteen of the dysplastic lesions progressed to oral SCC and 17 did not after several years of follow-up. Cathepsin L mRNA was overexpressed in 16/33 (48%) dysplastic lesions and in 9/14 (64%) oral SCC. Cathepsin L protein was also overexpressed in a large proportion of dysplasias and cancers. Overexpression was independent of dysplasia grade and identified in both those patients who progressed to oral SCC and in those who did not. Levels of cathepsin L mRNA and protein did not differ significantly in the progressing versus non-progressing dysplasias (P=0.27). However, cathepsin L mRNA and protein were significantly lower in the non-progressing dysplasias when compared to the oral cancers (P=0.03) but not in the progressing dysplasias suggesting a trend for dysplasias with overexpressed cathepsin L to be more likely to progress to oral cancer.


Leiomyosarcoma of the oral cavity is a very rare tumor that is associated with aggressive clinical behavior and low survival. In this paper, we report two new cases of leiomyosarcoma affecting the mandibular gingiva and mandible of a 35-year-old male and the mandible of a 51-year-old female. Given the difficulty in the histopathologic discrimination between benign and malignant smooth muscle tumors and the absence of reliable histologic parameters for prognostication of leiomyosarcomas, we evaluated the diagnostic and prognostic value of various immunohistochemical and molecular markers. By means of immunohistochemistry and quantitative real-time PCR analysis, we detected protein expression of PCNA, bcl-2, CDK4, p53...
and MDM2 in both our cases and MDM2 amplification in our second case. The literature, pertinent to oral leiomyosarcoma and to molecular analysis of smooth muscle tumors, is reviewed.


http://www.sciencedirect.com/science/article/B6TB6-4BSW5SW-B/2/0a509c1f60da3965c58b8180928fbf57

Squamous cell carcinoma of the upper aerodigestive tract (UADT) is associated with environmental factors, especially tobacco and alcohol consumption. Genetic factors, including cyclin D1 (CCND1) polymorphism have been suggested to play an important role in tumorigenesis and progression of UADT cancer. To investigate the relationship between CCND1 polymorphism on susceptibility for UADT cancers, 147 cancer and 135 non-cancer subjects were included in this study. CCND1 genotype at codon 242 (G870A) in exon 4 was undertaken using denaturing high performance liquid chromatography (DHPLC) and DNA sequencing. Significant odds ratio (OR) of the AA + GA genotypes [OR=7.5 (95% CI: 1.4-39.7)] was observed in non-drinkers but for non-smokers a non-significant [OR=5.4 (95% CI: 0.9-31.4)] was found in the adjusted model. These results suggest that allele A may be a risk factor for UADT cancer, especially in non-alcoholics. However, further epidemiological studies are needed to establish the exact role of CCND1 polymorphism and the development of UADT cancers.


http://www.sciencedirect.com/science/article/B6TB6-462BN8J-C/2/80d6f5e8870dfd9d0c848b424885302c

We undertook the genetic analysis of a classic Li-Fraumeni syndrome (LFS) family with clustering of primary tumours including two maxillary sarcomas, a rare LFS site of tumour occurrence. Our aim was to investigate the presence of a specific type of TP53 mutation that could be associated with this unusual predilection of site for cancer occurrence. Mutational screening of the coding region of TP53 revealed an A>T transversion in codon 144 of exon 5 (CAG>CTG, Gln>Leu) in the germline of one of the three affected members, with loss of heterozygosity (LOH) in the tumour tissue. All other affected members were negative for germline or somatic TP53 mutations. TP53 immunohistochemistry was uninformative. The mutation we report is a de novo constitutional TP53 mutation that has not been previously described in the literature. It could explain the more burdened phenotype of the affected patient (died at 21 months). Alternative mechanisms to explain the overall family phenotype are discussed.


http://www.sciencedirect.com/science/article/B6TB6-3W19WVP-3/2/db3b44673e394cab93d0d60d952c06d0

The inactivation of p53 tumour suppressor gene vis-a-vis point mutation, overexpression and degradation due to Human Papilloma virus (HPV) 16/18 infection, was examined in chewing
tobacco-associated oral cancers and oral leukoplakias from India. The analysis of mutations was assessed by polymerase chain reaction (PCR) with single strand conformation polymorphism (PCR-SSCP) of exons 5-9 on DNA from 83 oral cancer cases, and the mutations confirmed by direct nucleotide sequencing of the PCR products. p53 protein expression was evaluated by immunohistochemical analysis on paraffin-embedded sections of 62 representative oral cancer biopsies and 22 leukoplakias, using p53-specific monoclonal antibody DO-7. The presence of HPV16/18 was detected in the 83 oral cancer cases by PCR analysis using HPV L1 consensus sequences, followed by Southern hybridization with type-specific oligonucleotide probes. Forty-six per cent (38/83) of oral cancer tumours showed p53 alterations, with 17% (14/83) showing point mutations, 37% (23/62) with overexpression and 25% (21/83) with presence of HPV16 wherein the E6 HPV16 protein degrades p53. HPV18 was not detected in any of the samples. Ninety-two per cent concordance was observed between missense point mutations and overexpression of p53 protein. A significant correlation was not observed between p53 alterations in oral cancer and clinico-pathological profile of the patients. Twenty-seven per cent (6/22) of oral leukoplakias showed p53 overexpression. The overall p53 alterations in oral cancer tissues and oral lesions are comparable to data from the oral cancers reported in the Western countries with smoking and alcohol-associated oral cancers, and suggest a critical role for p53 gene in a significant proportion of oral cancers from India. The overexpression of p53 protein in leukoplakias may serve as a valuable biomarker for identifying individuals at high risk of transformation to malignant phenotype.


http://www.sciencedirect.com/science/article/B6TB6-45MVW76-C/2/521ba2037c848de7953c4ca4b200826b

In this study we performed p53 sequencing based mutation analysis in laryngeal cancers and matched recurrent disease following irradiation. The question is if irradiation affects the DNA and introduces or deletes mutations so that p53 cannot be used as a clonal marker anymore. P53 mutations were identified in fresh-frozen laryngectomy specimens with either primary laryngeal cancers, treated by surgery and irradiation post-operative with local failure during follow-up, or with recurrent laryngeal cancers following primary irradiation. In 21 tumors the p53 status was analyzed by direct sequencing full-length mRNA through RT-PCR. DNA sequencing analysis of exons 2 through 11 was performed when RNA isolation could not be performed. The marker mutation identified in this way was detected by DNA sequencing of the corresponding exon in formalin-fixed deparaffinized tumor biopsy samples in respectively matched recurrent disease following surgery and irradiation or primary tumor before irradiation. DNA sequencing analysis of the corresponding exon of peripheral blood leukocytes excluded the presence of germline mutations or polymorphisms. In 16 out of 21 tumors (71%), a mutation was identified. Fifteen of these marker mutations were detected in the matched tumor biopsy sample (94%). The only case lacking the marker mutation probably was a second primary tumor. We conclude that we find no direct evidence for induction or loss of p53 mutations following irradiation. Consequently, p53 may be used as a diagnostic tool when histological examination fails, for example in discriminating between the presence of a second primary tumor in the same area versus recurrent disease.


http://www.sciencedirect.com/science/article/B6TB6-3Y2MYWM-
Nasopharyngeal carcinoma (NPC) paraffin samples, from Spanish patients, of distinct histological types, including squamous cell carcinoma (10 cases), nonkeratinising carcinoma (12 cases) and undifferentiated carcinoma (29 cases) were analysed for Epstein-Barr virus (EBV) detection and EBV-encoded latent membrane protein (LMP-1) expression using a sensitive nested-polymerase chain reaction with four oligonucleotide primers specific for EBV genome (EB-1, 2, 3, 4) and immunohistochemistry by means of CS1-4 pool monoclonal antibody. EBV genome was detected regardless of histological type in 100% of samples with sufficient DNA quality to permit viral diagnosis (50 out of 51 cases), supporting the previous view that all types of NPC are variants of an EBV-associated malignancy. However LMP-1, an EBV-encoded oncogenic protein, was detected in 40 out of 51 samples (78.4%) and LMP-1 immunohistochemical expression was not apparently influenced by histological type, primary or metastatic site, clinical stage, age or sex. This high percentage of detection of LMP-1 in our cases supports a role for EBV in the pathogenesis of different types of NPC, but the lack of constant expression of LMP-1 in NPC remains unclear and various reasons are postulated to explain the absence of this oncogenic protein in some EBV-associated NPCs.

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics (1)


Although liposarcoma is one of the most common soft tissue sarcomas, its location in the oral cavity is very rare. To our knowledge, only 43 cases of liposarcoma originating in the oral tissues have been reported in the English-language literature. In this article, we report a case of well-differentiated liposarcoma affecting the cheek of a 28-year-old man and review the oral liposarcoma literature. Immunohistochemical analysis of the tumor revealed an MDM2+/CDK4+/p53+ immunophenotype that is consistent with the immunohistochemical profile of well-differentiated liposarcoma originating in other areas of the body. Quantitative polymerase chain reaction analysis of the DNA levels of the MDM2 (human homologue of the murine double-minute type 2), CDK4 (cyclin-dependent kinase 4), and SAS (sarcoma amplified sequence), genes was performed, revealing only SAS gene amplification. The possibility of misdiagnosis of oral liposarcoma because of its sometimes inconspicuous clinical and microscopic features is emphasized. Careful pathologic examination of liposarcoma is essential for discrimination from benign adipose tissue neoplasms and for precise histologic classification, both of major prognostic significance. Possible implications of molecular and cytogenetic analysis for unraveling the pathogenesis and determining the prognosis of liposarcoma are discussed. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:194-201)

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology (2)


ObjectiveTo investigate the prevalence of human papillomavirus (HPV) infection in normal oral mucosa, and to observe the natural history in the oral cavity in oral swab samples collected from healthy volunteers on Miyako Island, Okinawa, Japan.Study designThe prevalence of HPV infection in oral buccal mucosa cell scrapes collected between 2000 and 2002 from a cohort of 668 healthy volunteers was determined. HPV DNA was detected by consensus polymerase chain reaction (PCR) using MY09/MY11 primers followed by direct cycle sequencing. Just over 2 years later the HPV-positive participants were reevaluated.ResultsOf the 668 subjects, 662 samples were analyzed for HPV. HPV DNA was detected in 4 (0.6%) specimens. HPV type 16 (HPV16), HPV53, and HPV71, mucosal types, and HPV12, a cutaneous type, were all identified by direct sequencing. In the follow-up survey, the HPV71- and HPV12-positive participants again tested positive, while HPV DNA was not detected in the HPV16- and HPV53-positive participants.ConclusionThe results of this study among healthy individuals from Miyako Island suggest that oral HPV infection is uncommon. In this cohort, HPV71 and HPV12 were persistent, while HPV16 and HPV53 were transient in normal oral mucosa.


http://www.sciencedirect.com/science/article/B6WP1-4CRH670-3M/2/209dee1cb420efd8432505d1e57b8f3e

Objective. Warthin tumor of the salivary gland is composed of oncocytic epithelium with a prominent follicular lymphoid infiltrate. The purpose of this study was to characterize the clonality of this lymphoid component by means of polymerase chain reaction technology.Study design. DNA was isolated from paraffin-embedded tissue from 20 cases of typical Warthin tumor of the salivary gland and amplified by polymerase chain reaction to assess B- and T-cell clonality.Results. No dominant clonal populations were identified in any tumor. However, minor clonal expansions of both B and T cells were detected in up to 50% of tumors (immunoglobulin H, 50%; T-cell antigen receptor [beta], 10%; T-cell antigen receptor [gamma], 5%). No tumors showed evidence of bcl-2 proto-oncogene translocation, whereas 95% contained detectable Epstein-Barr virus DNA.Conclusion. The B- and T-cell components of Warthin tumor are polyclonal with oligoclonal expansion of both T and B cells in some lesions.

Organisms Diversity & Evolution (1)

Hexapoda includes 33 commonly recognized orders, most of them insects. Ongoing controversy concerns the grouping of Protura and Collembola as a taxon Ellipura, the monophyly of Diplura, a single or multiple origins of entognathy, and the monophyly or paraphyly of the silverfish (Lepidopteridae and Zygentoma s.s.) with respect to other dicondylous insects. Here we analyze relationships among basal hexapod orders via a cladistic analysis of sequence data for five molecular markers and 189 morphological characters in a simultaneous analysis framework using myriapod and crustacean outgroups. Using a sensitivity analysis approach and testing for stability, the most congruent parameters resolve Tricholepidion as sister group to the remaining Dicondyla, whereas most suboptimal parameter sets group Tricholepidion with Zygentoma. Stable hypotheses include the monophyly of Diplura, and a sister group relationship between Diplura and Protura, contradicting the Ellipura hypothesis. Hexapod monophyly is contradicted by an alliance between Collembola, Crustacea and Ectognatha (i.e., exclusive of Diplura and Protura) in molecular and combined analyses.

**Osteoarthritis and Cartilage (7)**


IntroductionArticular cartilage matrix synthesis and degradation are dynamic processes that must be balanced for proper maintenance of the tissue. In osteoarthritis (OA), this balance is skewed toward degradation and ultimate loss of matrix. The transcriptional and/or activity levels of hundreds of genes are dysregulated in chondrocytes from osteoarthritic cartilage, and a subset of these genes may represent pivotal factors that could be modulated if their specific role in the disease process could be identified. ObjectiveTo investigate the role of ADAMTS-4 and ADAMTS-5 in cartilage matrix degradation by developing a chondrocyte pellet culture assay in combination with adenoviral gene expression, and to demonstrate the utility of this assay by assessing the specific functional contribution of these genes to cartilage matrix metabolism. MethodsA full-length cDNA for bovine ADAMTS-4 (bADAMTS-4) was isolated, and used to evaluate the expression, regulation, and activity of this gene in bovine cartilage. Adenoviruses expressing bADAMTS-4, human ADAMTS-5 (hADAMTS-5) or human bone morphogenetic protein 2 (BMP-2) were used to infect primary chondrocytes, and their effect on extracellular matrix metabolism was assessed by monitoring the accumulation and release of glycosaminoglycans (GAG) in three-dimensional chondrocyte pellet cultures. ResultsAnalysis of bADAMTS-4 transcriptional regulation in chondrocytes revealed that interleukin-1[alpha] (IL-1[alpha]) was the most potent inducer of bADAMTS-4 mRNA and subsequent aggrecan degradation in cartilage explant cultures of those cytokines tested. bADAMTS-4 mRNA induction by IL-1[alpha] was greater in nasal cartilage than in articular cartilage. Chondrocytes infected with adenovirus expressing either bADAMTS-4 or hADAMTS-5 genes showed increased aggrecan degradation in newly synthesized matrix by pellet cultures while chondrocytes overexpressing BMP-2 showed increased aggrecan synthesis. ConclusionAdenoviral delivery of genes to primary bovine chondrocytes, followed by culture in three-dimensional pellet format and evaluation of extracellular matrix protein metabolism, is a useful functional assay for assessing the role of genes on cartilage matrix
Objective: We investigated how aging effects human chondrocyte yield, proliferation, post-expansion chondrogenic capacity, and response to specific growth factors supplemented during expansion.

Methods: Fifty-two samples of human articular cartilage were harvested from cadavers 20 to 91 years old and grouped into age decades. Cell yields were normalised to tissue wet weight. Cell proliferation rates were calculated during expansion in medium without (CTR) or with TGF[beta]1, FGF-2 and PDGF-BB (TFP). Chondrogenic capacity of CTR- and TFP-expanded cells was assessed by cultivation as 3D pellets in a defined serum-free medium, followed by histological, immunohistochemical, biochemical and real-time RT-PCR analyses.

Results: Cell yields were similar in donors up to 40 years of age and significantly lower (1.8-fold) in older donors. Cell proliferation rates in CTR medium significantly decreased after 30 years of age and remained similar in older donors. In the presence of TFP, proliferation rates were higher (up to 3.7-fold) in all age groups and decreased only slightly with age. The glycosaminoglycan (GAG) content of pellets obtained from CTR-expanded cells was not correlated with age. Pellets from TFP-expanded cells reproducibly contained more GAG (up to 3.2-fold) than those from CTR-expanded cells only if donors were younger than 40. Safranin O staining intensity and collagen type II expression and accumulation were consistent with GAG contents.

Conclusion: Medium supplementation with the growth factor combination TFP during chondrocyte expansion supports higher proliferation rates at any age and higher post-expansion chondrogenic capacity in donors up to 40 years. These findings may be relevant for chondrocyte-based cartilage repair procedures.


Summary: Objective The aim of this study was to develop high-throughput assays for the analysis of major chondrocyte functions that are important in osteoarthritis (OA) pathogenesis and methods for high-level gene expression and analysis in primary human chondrocytes. Methods In the first approach, complementary DNA (cDNA) libraries were constructed from OA cartilage RNA and full-length clones were selected. These cDNAs were transferred into a retroviral vector using Gateway Technology. Full-length clones were over-expressed in human articular chondrocytes (HAC) by retroviral-mediated gene transfer. The induction of OA-associated markers, including aggrecanase-1 (Agg-1), matrix metalloproteinase-13 (MMP-13), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), collagen IIA and collagen X was measured by quantitative real-time polymerase chain reaction (QPCR). Induction of a marker gene was verified by independent isolation of 2-3 clones per gene, re-transfection followed by QPCR as well as nucleotide sequencing. In the second approach, whole cDNA libraries were transduced into chondrocytes and screened for chondrocyte cluster formation in three-dimensional agarose cultures. Results Using green fluorescent protein (eGFP) as a marker gene, it was shown that the retroviral method has a transduction efficiency of >90%. A total of 40 verified hits were identified in the QPCR screen. The first set of 19 hits coordinately induced iNOS, COX-2, Agg-1 and MMP-13. The most potent of these genes were the tyrosine kinases Axl and Tyro-3, receptor interacting kinase-2 (RIPK2), tumor necrosis factor receptor 1A (TNFR1A), fibroblast growth factor (FGF)
and its receptor FGFR, MUS81 endonuclease and Sentrin/SUMO-specific protease 3. The second set of seven hits induced both Agg-1 and MMP-13 but none of the other markers. Five of these seven genes regulate the phosphoinositide-3-kinase pathway. The most potently induced OA marker was iNOS. This marker was induced 20-500 fold by seven genes. Collagen IIA was also induced by seven genes, the most potent being transforming growth factor [beta] (TGF[beta])-stimulated protein TSC22, vascular endothelial growth factor (VEGF) and splicing factor 3a. This screening assay did not identify inducers of collagen X. The second chondrocyte cluster formation screen identified 14 verified hits. Most of the genes inducing cluster formation were kinases. Additional genes had not been previously known to regulate chondrocyte cluster formation or any other chondrocyte function. Conclusions: The methods developed in this study can be applied to screen for genes capable of inducing an OA-like phenotype in chondrocytes on a genome-wide scale and identify novel mediators of OA pathogenesis. Thus, coordinated functional genomic approaches can be used to delineate key genes and pathways activated in complex human diseases such as OA.


http://www.sciencedirect.com/science/article/B6WP3-4BT1RSB-1/2/0c8ce162339a5a96d0b7a46f8bbdee40

Objective: To examine the capacity of recombinant osteogenic protein-1 (OP-1) to inhibit the cartilage extracellular matrix damage that follows treatment with hyaluronan hexasaccharides (HA6). Design: The effects of OP-1 were examined on isolated human chondrocytes grown in alginate beads as well as articular cartilage slices treated with hyaluronan hexasaccharides. Changes in the relative expression of messenger RNA for hyaluronan synthase-2, aggrecan and CD44 were determined by competitive quantitative reverse transcriptase-polymerase chain reaction. Cartilage proteoglycan biosynthesis was examined by a 35S-sulfate incorporation assay. Cell-associated matrix of human chondrocytes was visualized by the use of particle exclusion assay, and alcian blue staining. Cartilage slices were examined for accumulation of proteoglycan by Safranin-O, and hyaluronan by a specific biotinylated probe. Results: Combined OP-1 and HA6 treatment resulted in enhanced expression of mRNA for aggrecan and HAS-2, compared to the treatment with HA6 only. This increased expression of aggrecan mRNA was paralleled by an increased synthesis of cartilage proteoglycan especially retained in the cell-associated matrix. Co-treatment with OP-1 inhibited the HA6-induced depletion of cell-associated matrices as well as HA6-induced depletion of hyaluronan and proteoglycan within cartilage tissue slices. Conclusions: These results demonstrate that OP-1 can abrogate the catabolic events associated with a HA6-induced matrix depletion model of osteoarthritis. The mRNA levels of two major cartilage extracellular matrix components, aggrecan and hyaluronan synthase-2 are enhanced above values obtained by either OP-1 or HA6 treatments alone.


http://www.sciencedirect.com/science/article/B6WP3-4FK3P8P-1/2/dc3b4fc82a3cfeb636da995394748281cd

Summary: Objective To investigate the mechanism of aggrecanolysis in interleukin-1 (IL-1)-treated cartilage tissue by examining the time course of aggrecan cleavages and the tissue and medium content of membrane type 4-matrix metalloproteinases (MT4-MMP) and a disintegrin and metalloproteinase with thrombospondin type I motifs (ADAMTS).4. Methods: Articular cartilage
explants were harvested from newborn bovine femoropatellar groove. The effects of IL-1 treatment with or without aggrecanase blockade were investigated by Western analysis of aggrecan fragment generation, ADAMTS4 species (p68 and p53), and MT4-MMP, as well as by realtime PCR (polymerase chain reaction) for ADAMTS4 and 5. Aggrecanase was blocked with mannosamine (ManN), an inhibitor of glycosylphosphatidylinositol anchor synthesis, and esculetin (EST), an inhibitor of MMP-1, MMP-3, and MMP-13 gene expression. Results IL-1 treatment caused a major increase in MT4-MMP abundance in the tissue and medium. ADAMTS4 (p68) was abundant in fresh cartilage and this was retained in the tissue in untreated cartilage. IL-1 treatment for 6 days caused a marked loss of p68 from the cartilage and the appearance of p53 in the medium. Addition of either 1.35 mM ManN or 31-500 μM EST blocked IL-1-mediated aggrecanolysis and this was accompanied by nearly complete inhibition of the MT4-MMP increase, the p68 loss and the formation of p53. IL-1 treatment increased mRNA abundance for ADAMTS4 (~3-fold) and ADAMTS5 (~10-fold) but this was not accompanied by a marked change in enzyme protein abundance. Conclusion These studies support a central role for MT4-MMP in IL-1-induced cartilage aggrecanolysis and are consistent with the identification of p68 as the aggrecanase that cleaves within the CS2 domain, and of p53 as the aggrecanase that generates G1-NITEGE. Since the induction by IL-1 was not accompanied by marked changes in total ADAMTS4 protein, but rather in partial conversion of p68 to p53 and release of both from the tissue, we conclude that aggrecanolysis in this model system results from MT4-MMP-mediated processing of a resident pool of ADAMTS4 and release of the p68 and p53 from their normal association with the cell surface.


http://www.sciencedirect.com/science/article/B6WP3-48M7SCC-1/2/24f565be3c6fc166263e482daaa327d4

Objective: Certain forms of primary osteoarthritis (OA), particularly those affecting hand joints, have a genetic component. Recent studies have shown suggestive evidence that hand and knee OA are linked with the interleukin-1 (IL-1) region on human chromosome 2q. This study was undertaken to assess the association of primary OA of the hand (hand OA) with IL-1 region markers. Methods: Sixty-eight US Caucasoid cases and 51 US Caucasoid controls aged 60 years or older were recruited from the Mid-Atlantic region of the United States. Hand OA was classified by American College of Rheumatology (ACR) Clinical Criteria, and cases were subjected to radiographic examination for subgrouping. Genotyping was done for seven previously described single nucleotide polymorphisms (SNPs) of genes for IL-1[alpha] (encoded by IL1A), IL-1[beta] (IL1B), and the IL-1 receptor antagonist (IL1RN), as well as an IL1RN variable number of tandem repeat (VNTR) marker. Six microsatellite markers on other chromosomes (null loci) were also typed. Results: The IL1B 5810 G>A SNP genotypes marker were not in Hardy-Weinberg equilibrium (p=0.007). This IL1B 5810 AA genotype association was also significant between erosive and non-erosive hand OA subjects (relative risk 4.01, p=0.008). As expected, significant linkage disequilibrium was present between IL1B 5810 SNP and IL1A (-)889 SNP, other IL1B SNPs, and the nearest IL1RN SNP examined. The IL1B 5810A allele occurs most frequently on haplotypes with the SNP alleles IL1B 1423C, IL1B 1903T, IL1B 5887C, and IL1A (-)889C. Genotypes at null loci failed to show evidence suggesting population stratification that might account for spurious association. Conclusion: Statistical evidence shows association between erosive hand OA and a genomic region containing the IL1B 5810 SNP in a US Caucasian population. This supports a potential role for IL-1 in the pathogenesis of a severe phenotype of hand OA.
http://www.sciencedirect.com/science/article/B6WP3-4FRJKX7-3/2/b234f77a406c40d5c85354dd29e4fbd

Summary
Objectives: To elucidate the antagonism between interleukin-1 (IL-1) and transforming growth factor-[beta] (TGF-[beta]) at the gene expression level, as IL-1 and TGF-[beta] are postulated to be critical mediators of cartilage degeneration/protection in rheumatic diseases.
Methods: The H4 chondrocyte cell line was validated by comparing metalloproteinase expression profile with intact murine cartilage by reverse transcription polymerase chain reaction. Genome-wide gene expression in the H4 cells in response to IL-1 and TGF-[beta], alone and in combination, was analyzed by using oligonucleotide arrays negotiating approximately 12,000 genes.
Results: The response of cartilage and the H4 cell line to IL-1 and TGF-[beta] was comparable. Oligonucleotide array analysis demonstrated a mutual but asymmetrical antagonism as the dominant mode of interaction between IL-1 and TGF-[beta]. Cluster analysis revealed a remarkable selectivity in the mode of action exerted by TGF-[beta] on IL-1 regulated genes: antagonistic on pro-inflammatory genes whereas additive on growth regulators such as vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF). While the former cluster underlined the protective effect of TGF-[beta], the latter underscored the adverse effect of TGF-[beta]. We further identified potentially novel classes of target genes under control of TGF-[beta] such as ras family, histones, proteasome components, and ubiquitin family, highlighting the importance of such genes in TGF signaling besides the well-characterized SMAD pathway.
Conclusions: We identified a cluster of genes as potential targets mediating the adverse effect of TGF-[beta] such as fibrosis. Transcriptional regulation of ras GTPase and ubiquitin/proteasome pathways is likely to be a novel mechanism mediating the effect of TGF-[beta] and its interaction with IL-1. These down-stream genes and pathways can be targets in future therapy.

http://www.sciencedirect.com/science/article/B6T0K-4CGN1WH-1/2/b18622b3de2c34f1f4044f239abba3ce

Tissue damage during surgery can induce 'central sensitization' and the development of pain and hyperalgesia post-operatively. Metabotropic glutamate receptors (mGluRs) contribute to nociception, inflammatory pain and hyperalgesia. This study characterized the temporal expression of group I (mGluR1, mGluR5) and II (mGluR2, mGluR3) mGluRs in spinal cord following abdominal surgery. Lumbar spinal cord was recovered from adult sheep euthanased 5 h, 1, 2, 3 and 6 days after undergoing a midline laparotomy, and processed for mGluR mRNA (real-time PCR, in situ hybridization) and protein (Western blotting). mGluR5 mRNA was up-regulated 5 h and 1 day post-surgery in laminae I-II of the spinal cord dorsal horn. mGluR5 protein was increased 1 day post-surgery. A delayed induction of mGluR2 and mGluR3 mRNAs and mGluR2/3 protein occurred in spinal cord 3 days after surgery. By 6 days, mGluR2 mRNA levels had returned to normal, however, mGluR3 mRNA and mGluR2/3 protein remained elevated. No change was detected in mGluR1. These results demonstrate that mGluRs are differentially regulated following surgery and support a link between mGluR-mediated activity and post-surgical pain.
Evidence from experimental pain research has revealed that metabotropic glutamate receptors (mGluRs) play a pivotal role in nociceptive processing, inflammatory pain and hyperalgesia. The aim of this study was to characterise expression of group I and II mGluRs in spinal cord in a model of naturally occurring persistent inflammation (sheep with unilateral lameness due to inflammation of the digital tissues of the feet, estimated to have been affected by the condition for >2 weeks) and an experimental model of acute inflammation (injection of intradermal carrageenan into lower forelimb in sheep). Animals with unilateral clinical inflammation displayed significant mechanical hyperalgesia on the affected limb. Carrageenan treatment produced significant bilateral limb mechanical hyperalgesia 3 h post-injection. Up-regulation of mGluR3 and mGluR5 mRNA was observed in ipsilateral spinal cord recovered from clinically lame animals, restricted to laminae II-V and I-II, respectively. Western blot analyses of protein extracts revealed a bilateral increase in mGluR2/3 and mGluR5. No change was detected in spinal cord mGluR1 or mGluR2 mRNA. There was no change in mGluR1,2,3,5 subtype mRNA or proteins in spinal cord recovered from animals 3 h post-carrageenan. These results demonstrate for the first time that mGluR subtypes are differentially expressed in spinal cord dorsal horn in response to persistent inflammation, and suggest that mGluR activity may be involved in mediating altered behaviours associated with clinical inflammatory pain.

Tumor necrosis factor [alpha] (TNF[alpha]) and interleukin 1[beta] (IL-1[beta]) are pro-inflammatory cytokines capable of altering the sensitivity of sensory neurons. Because sensitization elicited by IL-1[beta] and TNF[alpha] is blocked by inhibition of the inducible enzyme, cyclooxygenase-II (COX-2), we examined whether these cytokines could increase COX-2 expression in dorsal root ganglion (DRG) cultures. Treatment of cell cultures with either IL-1[beta] or TNF[alpha] increases immunoreactive COX-2, as measured by immunoblotting, in a time- and concentration-dependent manner. A 24-h pretreatment with 10 ng/ml IL-1[beta] or 50 ng/ml TNF[alpha] augmented COX-2 expression 50- and 8-fold over basal levels, respectively. Immunohistochemistry established the presence of COX-2-like immunoreactivity in both neuronal and non-neuronal cells in culture. The addition of IL-1 receptor antagonist blocked the induction of COX-2 expression by IL-1[beta], but did not alter TNF[alpha]-stimulated increases in COX-2, indicating that the mechanism of TNF[alpha] is not limited to increasing the expression of IL-1[beta]. The basal and TNF[alpha]-induced expression of COX-2 was not dependent on the presence of NGF in the growth media. IL-1[beta] and TNF[alpha] treatment for 24 h enhanced prostaglandin E2 (PGE2) production 2-4-fold, which was blocked by pretreatment with the COX-2 inhibitor, NS-398. Exposing cultures to PGE2, IL-1[beta], or TNF[alpha] for 24 h did not alter PGE2 receptor (EP) mRNA levels. These results indicate that TNF[alpha] and IL-1[beta] induce the functional expression of COX-2 but not EP receptors in DRG cells in culture and suggest that cytokine-induced sensitization of sensory neurons is secondary to prostaglandin production and not alterations in EP receptors.
To determine which \([\alpha]2\)-adrenergic receptor subtypes are present in primary afferent and sympathetic postganglionic neurons we have performed in situ hybridization and immunohistochemistry experiments on rat dorsal root and superior cervical ganglia. Reverse transcriptase polymerase chain reaction was used as a preliminary screen for the presence of mRNA encoding \([\alpha]2\)-adrenergic subtypes in dorsal root and superior cervical ganglia; polymerase chain reaction primers amplified distinct regions of the rat \([\alpha]2A\) (RG20), \([\alpha]2B\) (RNG) and \([\alpha]2C\) (RG10) adrenergic receptor subtypes in mRNA extracted from lumbar dorsal root and superior cervical ganglia. To localize receptors to cell types in the ganglia, in situ hybridization was performed on cryosections of dorsal root and superior cervical ganglia with oligonucleotide probes designed to distinguish between mRNA encoding for \([\alpha]2\)-adrenergic receptor subtypes. Immunohistochemistry was performed with a polyclonal antibody against the \([\alpha]2A\)-adrenergic receptor subtype. Our results with reverse transcriptase polymerase chain reaction indicate that all three \([\alpha]2\)-adrenergic receptor subtypes are expressed in dorsal root and superior cervical ganglia. Data from the in situ hybridization experiments indicated that the mRNA detected with the reverse transcriptase polymerase chain reaction was present in neuronal cell bodies, except for the mRNA encoding the \([\alpha]2A\)-adrenergic receptor which was not detectable in dorsal root ganglia. The distribution of mRNA encoding \([\alpha]2B\)- and \([\alpha]2C\)-adrenergic receptor subtypes among dorsal root ganglion neurons and \([\alpha]2A\)-, \([\alpha]2B\)- and \([\alpha]2C\)-adrenergic receptor subtypes among superior cervical ganglion neurons suggests that multiple adrenergic receptor subtypes are present in a single neuron. Neuronal cell bodies in both the dorsal root and superior cervical ganglion consistently demonstrated \([\alpha]2A\)-adrenergic receptor-like immunoreactivity. The apparent co-expression of multiple \([\alpha]2\)-adrenergic receptor subtypes in dorsal root and superior cervical ganglion neurons enables a single transmitter to produce a number of effects in the same neuron; which receptors are functionally active may vary with the presence of nerve injury, inflammation or other physiological and pathophysiological conditions.

Transcriptional changes evoked in nociceptive sensory neurons by inflammatory injury play a substantial role in the generation of and recovery from painful hypersensitivity. Transgenic mice overexpressing nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF) in the skin possess a greatly increased number of nociceptors. Surprisingly, NGF-overexpressers display reduced hypersensitivity and recovered more rapidly in response to inflammation, suggesting a compensatory suppression of nociceptive transmission in these mice. To determine whether these transgenic mice show changes in inflammation-evoked transcriptional plasticity, we examined the expression of a panel of genes implicated in nociceptive signaling in response to injection of complete Freund’s adjuvant into the hindpaw. Relative mRNA levels were quantified 1, 4 and 15 days after injection using real-time PCR. In wild type mice CFA injection elicited a reproducible pattern of altered gene expression that returned to baseline over a 2-week period. In mice overexpressing NGF or GDNF the expression patterns for several genes were substantially altered; these changes in injury-evoked patterns of gene expression suggest the
existence of endogenous regulatory mechanisms that can compensate for increased nociceptive input by modulating the expression of a limited subset of genes.


http://www.sciencedirect.com/science/article/B6T0K-45G037C-2/2/836218c927ff22eaf93dbc1d967e27a4

A variety of molecules released by inflammatory reactions in the dorsal root and dorsal root ganglion (DRG) may play important roles in the pathology of neuronal abnormalities in lumbar disc herniation. In order to elucidate the pathophysiological mechanisms of painful radiculopathy, secondary to lumbar disc herniation, we evaluated pain-related behavior and the change of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) expression in the DRG and dorsal root using a rat model of lumbar disc herniation. In the nucleus pulposus (NP) group, the left L4/5 nerve roots were exposed after hemilaminectomies and autologous intervertebral discs, which were obtained from coccygeal intervertebral discs, were implanted on each of the exposed nerve roots without mechanical compression. Rats in the NP group, but not the sham-operated rats, developed mechanical allodynia on the ipsilateral hind paw for 1 day after surgery and showed a significant increase in the number of NGF-immunoreactive (IR) cells in the nerve root and DRG. NGF-IR cells in the nerve root and DRG included macrophages and Schwann cells, because these cells were labeled for NGF and ED-1 or glial fibrillary acid protein by dual immunostaining. A significant increase in the percentage of BDNF-IR neurons in the DRG was observed in the NP group at 3 days after surgery and the increase in BDNF mRNA expression was confirmed using in situ hybridization histochemistry and reverse transcription-polymerase chain reaction. We also injected NGF into the endoneurial space of the normal rat spinal nerve root and found that the NGF injection produced dose-dependent mechanical allodynia on the ipsilateral hind paw at 1 day after surgery and an increase in the percentage of BDNF-IR neurons in the DRG at 3 days after surgery compared to the group receiving saline injection. These findings suggest that in the lumbar disc herniation model, i.e. neuritis of the nerve root, increased NGF produced by the inflammatory responses in the dorsal root and DRG tissues may affect the production of BDNF in the DRG and may play important roles in the modulation of the dorsal horn neurons. These changes in neurotrophic factors in the primary afferents may be involved in the pathophysiological mechanisms of neuropathic pain produced by lumbar disc herniation.


http://www.sciencedirect.com/science/article/B6T0K-45GKR5N-2/2/8ca5641301895ec620c0dbdcb8f66f56

One of the major serotonin (5-HT) receptor subtypes expressed in the rat dorsal root ganglion (DRG) neurons is the 5-HT2A receptor. We have previously shown that 5-HT2A receptors in the peripheral sensory terminals are responsible for 5-HT-induced pain and hyperalgesia. In the present study, we characterized neurons expressing 5-HT2A receptors in the rat DRG neurons by means of in situ hybridization, immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR) and behavioral tests. In situ hybridization on consecutive sections revealed that 5-HT2A receptor mRNA is colocalized with calcitonin-gene related peptide (CGRP) mRNA (100/104; 96.2%) but not with c-Ret mRNA (1/115; 0.9%). Signals for 5-HT2A receptor mRNA were found in 9.4+/-2.2% of normal DRG (L5) neurons, most of which were small to medium in size. Four days of complete Freund's adjuvant-induced inflammation of the hindpaw doubled the incidence of 5-HT2A receptor mRNA-expressing neurons to 19.3+/-2.8%. The level of 5-HT2A
receptor mRNA in DRGs of normal and various pathological conditions was then determined by RT-PCR. The level was up-regulated by peripheral inflammation, but not by axotomy or chronic constriction of the peripheral nerve. Systemic administration of 5-HT2A receptor antagonist (Sarpogrelate HCl) produced analgesic effects on thermal hyperalgesia caused by peripheral inflammation, but failed to attenuate thermal hyperalgesia in chronic constriction injury model. These findings suggest that 5-HT2A receptors are mainly expressed in CGRP-synthesizing small DRG neurons and may be involved in the potentiation of inflammatory pain in the periphery.


http://www.sciencedirect.com/science/article/B6T0K-4DDXVX0-4/2/30fd6192ecd44e60aba3b5fc56d0fbb

Intrathecal (i.t.) injection of nociceptin elicited a behavioral response mainly consisting of biting and licking, which were eliminated by the i.t. co-administration of opioid receptor-like-1 (ORL-1) receptor antagonists. The behavioral response induced by nociceptin was characteristically similar to that by i.t.-administered histamine, and was attenuated by i.t. co-administration of the H1 receptor antagonists, but not by the H2 receptor antagonists, whereas the H3 receptor antagonist promoted the nociceptin-induced behavior. H1 receptor knockout (H1R-KO) mice did not show the nociceptin-induced nociceptive behavior, which was observed in wild-type mice. Pretreatment with a histamine antiserum or a histidine decarboxylase inhibitor resulted in a significant reduction of the response to nociceptin. The previous studies showed that NK1 receptor antagonists and a novel substance P (SP)-specific antagonist given i.t. could reduce the behavioral response to nociceptin and histamine. On the other hand, the nociceptive response induced by nociceptin, but not histamine, was completely attenuated by the i.t. co-administration of agonists for GABAA and GABAB receptors. In contrast, the antagonists for GABAA and GABAB receptors injected i.t. showed same nociceptive response with nociceptin and histamine, and their nociceptive responses were significantly blocked by the i.t. co-administration of the H1 receptor antagonists, but not H2 receptor antagonists or ORL-1 receptor antagonists. The present results suggest that the activation of the ORL-1 receptor by nociceptin may induce the disinhibition of histaminergic neuron and enhance the release of histamine, which subsequently acts on the H1 receptor located on the SP-containing neurons to produce the spinal cord-mediated nociceptive response.


http://www.sciencedirect.com/science/article/B6T0K-42MFFR7-3/2/1ac95477d2c6e973712291731b4cb57d

Recent theories of pathogenesis of pain in chronic pancreatitis (CP) are neuroimmune interactions of intrapancreatic nerves and inflammatory cells and increase in levels of pain neurotransmitters such as substance P (SP). This study analyzed the expression and localization of neurokinin 1 receptor (NK-1R), which binds SP, and its association with pain and inflammation in CP. Pancreatic tissues from 31 patients (22 males, nine females; mean age 45.9+/−9.4 years) with CP were evaluated. Nine normal pancreases (five males, four females; mean age 42.9+/−9.5 years) served as controls. Quantitative PCR was used to determine the NK-1R mRNA expression levels and in situ hybridization and immunohistochemistry were used to localize expression sites of NK-1R mRNA and protein, respectively. We also analyzed whether an association exists between NK-1R mRNA expression and pain and inflammation. In CP samples, in situ hybridization and immunohistochemistry localized NK-1R mRNA expression and protein mainly in
the nerves, ganglia, blood vessels, inflammatory cells and occasionally in fibroblasts. In patients with mild to moderate and strong intensity of pain, NK-1R mRNA levels were increased 14- and 30-fold over controls, respectively. There was a significant relationship between NK-1R mRNA levels and intensity of pain (r=0.46, P=0.03), NK-1R mRNA and the frequency of pain (r=0.51, P=0.04), and NK-1 mRNA and duration of pain (r=0.46, P=0.01) in CP patients, but not with the degree of tissue inflammation. NK-1R signaling may be involved in the pain syndrome of CP. The expression of NK-1R in inflammatory cells and blood vessels also points to an interaction of immunoreactive substance P nerves, inflammatory cells and blood vessels, and further supports the existence of a neuroimmune interaction that probably influences the pain syndrome and chronic inflammatory changes so characteristic of CP.


http://www.sciencedirect.com/science/article/B6T0K-4BM8KGG-1/2/a846d3f760d79f30e36ada5500a0c651

Based on a hypothesis that interleukin 1 (IL-1) activity is associated with low back pain (LBP), we investigated relationships between previously described functional IL-1 gene polymorphisms and LBP. The subjects were a subgroup of a Finnish study cohort. The IL-1[alpha](C889-T), IL-1[beta](C3954-T) and IL-1 receptor antagonist (IL-1RN)(G1812-A, G1887-C and T11100-C) polymorphisms were genotyped in 131 middle-aged men from three occupational groups (machine drivers, carpenters and office workers). A questionnaire inquired about individual and lifestyle characteristics and the occurrence of LBP, the number of days with pain and days with limitation of daily activities because of pain, and pain intensity, during the past 12 months. Lumbar disc degeneration was determined with magnetic resonance imaging. Carriers of the IL-1RNA1812 allele had an increased risk of LBP (OR 2.5, 95% CI 1.0-6.0) and carriers of this allele in combination with the IL-1[alpha]T889 or IL-1[beta]T3954 allele had a higher risk of and more days with LBP than non-carriers. Pain intensity was associated with the simultaneous carriage of the IL-1[alpha]T889 and IL-1RNA1812 alleles (OR 3.7, 95% CI 1.2-11.9). Multiple regression analyses allowing for occupation and disc degeneration showed that carriage of the IL-1RNA1812 allele was associated with the occurrence of pain, the number of days with pain and days with limitations of daily activities. Carriage of the IL-1[beta]T3954 allele was associated with the number of days with pain. The results suggest a possible contribution of the IL-1 gene locus polymorphisms to the pathogenesis of LBP. The possibility of chance findings cannot be excluded due to the small sample size.


http://www.sciencedirect.com/science/article/B6T0K-487F009-3/2/a4156b6570f2e60eaa9e05f44de4ebb7

An association between HLA-DR13 and patients with complex regional pain syndrome (CRPS) who progressed towards multifocal or generalized tonic dystonia was recently reported. We now report on a new locus, centromeric in HLA-class I, which was significantly associated with a spontaneous development of CRPS, suggesting an interaction between trauma severity and genetic factors conferring CRPS susceptibility. Additionally, an association with the D6S1014 locus was found, supporting the previous finding of an association with HLA-DR13.

http://www.sciencedirect.com/science/article/B6T0K-4B0WSF5-3/2/88fead1d7052e2f17ede7d8a43e508ef

At least two classes of nociceptors can be distinguished based on their growth factor requirements: glial cell-line derived neurotrophic factor (GDNF)- and nerve growth factor (NGF)-dependent primary afferent neurons. Based on numerous anatomical and biochemical differences, GDNF- and NGF-dependent neurons have been proposed to be involved in the development of different types of persistent pain. To examine this hypothesis we used two lines of transgenic mice that contained a supernormal number of either NGF- or GDNF-dependent neurons (referred to as NGF-OE and GDNF-OE mice, respectively). These mice were tested in a model of inflammatory pain (induced by injection of complete Freund's adjuvant) and neuropathic pain (using a spinal nerve ligation protocol). Contrary to expectations, neither line of transgenic mice became more hyperalgesic following induction of persistent pain. In fact, NGF-OE mice recovered more rapidly and became hypoalgesic despite extensive paw swelling in the inflammatory pain model. In the neuropathic pain model, only wildtype mice became hyperalgesic. Real-time PCR analysis showed that the NGF-OE and GDNF-OE mice exhibited changes in neuronal-specific mRNAs in the dorsal root ganglia but not the spinal cord dorsal horn. These results indicate that increasing the number of nociceptors results in potent compensatory mechanisms that may begin with changes in the sensory neurons themselves.

Parasitology International  (5)


http://www.sciencedirect.com/science/article/B6TB7-44V20RR-1/2/c4240505ef2cd6894961cc16d41d6bca

It has been reported that Leishmania promastigotes have ability to express foreign genes on drug selectable plasmids. To investigate further abilities of the recently described expression vector, P6.5, in the transfection of Leishmania organisms (Chen D-Q, Kollì BK, Yadava N et al. Episomal expression of specific sense and antisense mRNAs in Leishmania amazonensis: modulation of gp63 levels in promastigotes and their infection of macrophages in vitro. Infect Immun 2000;68:80-86), the constructed expression vector, which contains canine interleukin-8 (cIL-8) coding cDNA, was introduced by electroporation to promastigotes of four species of the genus Leishmania: Leishmania amazonensis, L. equatorensis, L. donovani and L. infantum. Extrachromosomal DNAs and total RNAs from the transfected promastigotes were subjected to polymerase chain reaction (PCR) and reverse transcriptase-PCR, respectively, using cIL-8 gene specific primers, and a predicted product of 330 bp was detected. Western blot analysis using a mouse monoclonal antibody raised against cIL-8 demonstrated the successful expression of cIL-8 in the transfectants and culture supernatants. Culture supernatants of the transfected L. amazonensis and L. equatorensis promastigotes showed a high chemotactic activity to both dog and mouse polymorphonuclear leukocytes. These results indicate that Leishmania promastigotes transfected with the expression vector P6.5 containing cIL-8 cDNA are capable of producing biologically active cIL-8. The Leishmania expression system using the P6.5 vector might be a
useful alternative for the production of biologically active recombinant cytokines.


http://www.sciencedirect.com/science/article/B6TB7-4BRJH86-3/2/d1a8c12abb30a5a026376dcc12df00d

Thermophilic amoeboflagellates in the genus Naegleria include both virulent and benign species. One of the less studied species, N. italica, has not been detected in the environment since the first reports from Italy in the 1980s; its virulence is known only from infection of laboratory mice. Two recent strains from recreational water in Western Australia (AWQC NG960, NG961) were tentatively identified as N. italica from the characteristic mobilities of seven isozymes. Sequences of the 5.8S rRNA gene and its flanking ITS aligned with a 380+bp length of the published sequence for N. italica with 98% identity. Differences from the type strain were confined to ITS2. Shorter alignments (Naegleria species, corresponding to conserved regions of the 5.8S gene and ITS. Unlike the European type strain of N. italica, the Australian isolates failed to infect laboratory mice intranasally, confirming that infectivity of this species is variable and often lower than in N. fowleri.


http://www.sciencedirect.com/science/article/B6TB7-47T8SF7-1/2/cf103bd509b085de7321772035787d14

The amino-terminal region of the serine repeat antigen (SERA) of Plasmodium falciparum is a major malaria-vaccine candidate. Variation in this molecule is essentially dimorphic and alleles may be grouped into the types FCR3, K1 and Honduras1. The Honduras1-type is thought to be the product of homologous recombination between FCR3 and K1 alleles. Here we have examined patterns of sequence diversity in exon II of SERA gene, which encodes most of the amino-terminal region of the antigen, in wild P. falciparum isolates from Indonesia (n=60), Myanmar (n=10) and Thailand (n=14). Among the Indonesian isolates the FCR-3 type predominated (56/60), twenty of which we characterized as novel alleles. A new K1-type allele was also found. In Myanmar, however, all isolates displayed K1-type SERA sequences, which included one new allele. The Honduras1-type was not detected in both countries. In contrast, the 14 isolates from Thailand displayed all three allelic types, with one new Honduras1-type and three new K1-type alleles. On examining the global distribution of SERA alleles by combining previously published sequence data with our results, the FCR3-type alleles predominated in Indonesia, Brazil, and Solomon Islands, but were not found in wild isolates from Myanmar and Africa. Brazil was the only area where K1-type alleles were not found. The distribution of Honduras1-type alleles seems to be mostly restricted to parasite populations from Vietnam, Thailand and Africa. In the allelic families FCR3 and K1, most diversity resulted from variation in sequence and number of octamer repeat units and of allotypes encoding the stretch of serine residues. Sequence analysis indicated that both insertions and deletions of repetitive motifs (creating variation within dimorphic allelic families) and homologous recombination between alleles belonging to different allelic families (creating Honduras1-type alleles) play a role in generating new SERA alleles. Since repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitory antibodies, sequence variation in exon II may represent one of the parasite’s immune-evasion strategies.

http://www.sciencedirect.com/science/article/B6TB7-4BFVSG0-2/2/0f823b2de04a4a987069bb9efc318a66

The mechanisms producing the genetic polymorphism at Plasmodium falciparum merozoite surface antigen-1 locus (pfmsp1) include the insertion and deletion of the different type of dimorphic Block 2 9-nucleotide repeat units as well as the intragenic recombination. To study relative occurrence frequencies of these two distinct mechanisms, we have developed a sensitive PCR strategy to identify both 5’ recombinant types and the number of Block 2 repeats from the same sample. This method can specifically detect the target 5’ recombinant type (Blocks 2-6) at the sensitivity of 1-4 copies of the pfmsp1. Applying the new method to field isolates from the Solomon Islands enabled us to identify six different 5’ recombinant types and variation in Block 2 repeat number in three of them, thus distinguishing 10 different alleles. Distribution of these alleles in local three villages in the study area suggests that frequencies of variation in the number of Block 2 9-bp repeats and recombination events within Blocks 2-6 are mutually independent and the frequency of repeat variation is relatively high as compared to that of recombination events at the pfmsp1 locus in P. falciparum populations from the Solomon Islands.


http://www.sciencedirect.com/science/article/B6TB7-4CCCP61-3/2/5765c3cfe9f0562838df3c77f36b5883

The most frequently used antimitotic agent in cytogenetic studies is colchicine. We investigated whether the initial treatment of trematodes for karyological analysis with colchicine would have mutagenic or degradational effect on rDNA sequences. Dreissena polymorpha is the intermediate host of Phyllodistomum folium and Bucephalus polymorphus, and the sporocyst stage of these trematode species develop, respectively, in the gills and gonads of this mussel. Sporocysts of P. folium and B. polymorphus were obtained from D. polymorpha collected from waterbodies in Belarus and in Lithuania. 5.8S and 28S rDNA genes, ITS1 and ITS2 of P. folium and B. polymorphus were sequenced and compared, and no nucleotide sequence differences between colchicine treated and untreated trematodes were found. Based on these results, we conclude that colchicine treatment for 3-5 h has no mutagenic or degradational effect on rDNA sequences. During the course of this investigation, two genetically different P. folium samples were noted in Belarus.

Parkinsonism & Related Disorders (2)

Exonic deletions of the Parkin gene are common in the autosomal recessive form of juvenile parkinsonism. Here we report Parkin gene mutations among apparently sporadic Parkinson's disease (PD) patients. We screened 200 patients with PD (103 women and 97 men). The age of onset was 54.2 +/- 10.3 years (mean +/- S.D.). Four out of the 200 patients had homozygous exonic deletions in the Parkin gene. The clinical features of these four patients were essentially the same as those of idiopathic PD. The age of onset was consistently younger (33, 38, 47 and 48 years, respectively). On medication, all of them were at Hoehn and Yahr stage II or III even after 12-16 years from the onset of the disease. Thus 2% of apparently sporadic PD patients in Japan have homozygous Parkin gene mutations. This positive rate was 6.3% among the patients with the age of onset below 50. Our study suggests that the prevalence of the carrier state of Parkin gene may be more than that we expected. Our study warrants further studies on Parkin gene mutations in apparently sporadic PD patients.


Early Onset Parkinson's Disease (EOPD) is characterized by selective degeneration of nigrostriatal dopaminergic neurons and a marked response to levodopa. However, at present, few methods are available as diagnostic tools for EOPD except for 18F-DOPA PET. In addition, little is known about the correlation between clinical severity, neuroimaging grading and genetic susceptibility. In the present study, 99mTc-TRODAT-1 SPECT and brain MRI were used to identify 30 cases of non-familial EOPD from a Chinese cohort of 230. All 30 PD patients had an age of onset of less than 55 years (mean age at onset, 41.5[plus-or-minus sign]9.3 years). Each of the 30 EOPD cases was sub-classified into one of five stages based on the 99mTc-TRODAT-1 SPECT findings. In the early stages of PD (stages 1 and 2), a lower uptake of 99mTc-TRODAT-1 in the putamen was found, while uptake in the caudate nucleus was normal. In the latter stages (stages 3, 4, 5), 24 patients revealed a diffuse and uniform loss of 99mTc-TRODAT-1 uptake in the putamen and the caudate nucleus. Further, in conventional genetic studies of the 30 patients, six novel mutations were found in the Parkin gene, and these included five heterozygous point mutations (C441R, Q311H, V258M, C212G, and S193I) and one homozygous deletion (exon 10-12). Known polymorphisms (Ser167Asn, Val380Leu) were also found in a number of patients. However, gene dosage analysis did not reveal any compound heterozygous mutations in these 30 patients using quantitative duplex PCR. This is the first study to examine EOPD patients of Chinese ethnic background (not exhibiting a definite familial trait), to offer a complete genetic analysis of the Parkin gene, and to correlate clinical stages of the disease with dopamine re-uptake.

Pathology - Research and Practice (3)

The detection of tumor cells in the sentinel lymph node (SLN) is of great importance for the prognosis of cancer patients. At present, immunohistochemistry and RT-PCR for tumor marker expression are the most sensitive techniques available for this analysis. However, so far, most RT-PCR-based analyses of SLNs have been performed on fresh material, excluding a direct comparison with the (immuno)histologic results. In our view, this does not entirely aid routine diagnosis. We established an efficient method for RNA extraction and RT-PCR from paraffin sections of SLNs from prostate cancer patients and compared the results with the (immuno)histologic data of adjacent sections. Amplifiable RNA was obtained from 133 SLNs of 68 prostate cancer patients. Correlation of PSA-specific RT-PCR with (immuno)histologic findings showed a positive and negative predictive value of 83% and 100%, respectively, for the prostate cancer patients investigated. Four of 12 patients with biochemical relapse, but without (immuno)histologically detectable tumor cells were RT-PCR-positive for PSA. We found that single sections of paraffin-embedded SLNs are suitable for routinely performed RT-PCR. Combined with (immuno)histology, PSA-specific RT-PCR is a revealing supplementary technique for the detection of tumor cells in SLNs of prostate cancer patients.


Amplification and overexpression of the HER-2/neu (c-erbB-2) oncogene have been observed in many cancers and are associated with a poor prognosis particularly in breast cancer. The human epidermal growth factor (HER)-2 receptor has recently been implicated in Ewing's sarcoma tumor cell line growth and chemosensitivity. The present study evaluates the amplification of HER-2/neu gene in paraffin sections from 42 cases of Ewing's sarcoma by a real-time quantitative polymerase reaction method using LightCycler system (Roche diagnostics, GmbH Mannheim, Germany). The relative copy number of HER-2/neu versus [beta]-globin was calculated at the crossing point. The mean calculated copy number in these cases of Ewing's sarcoma and normal controls was 26.43 and 26.93, respectively. The p value was 0.215 (p). Our results demonstrated an absence of HER-2/neu oncogene amplification in Ewing's sarcomas, and we suggest that HER-2/neu is not a biologically or therapeutically important pathway in Ewing's sarcoma.


Low-grade central osteosarcoma is an uncommon form that is characterized by a long premorbid history, and is compatible with prolonged survival after treatment. However, molecular abnormalities are rare in low-grade central osteosarcomas, whereas p53 mutations occur in approximately 20% of conventional high-grade osteosarcomas. In this study, 21 cases of low-grade central osteosarcoma were analyzed for mutations of the p53 gene, amplification of the MDM2 gene, and mutations of the H-ras gene using formalin-fixed, paraffin-embedded materials. We also examined the expression of p53, MDM2, and p21WAF1 protein immunohistochemically...
and assessed the proliferation activities using the monoclonal antibody MIB-1. One case (4.7%) showed strong p53 immunoreactivity, whereas p53 gene mutations were not detected at all. Seven cases (33.3%) showed immunoreactivity for MDM2 protein. As for gene alterations, MDM2 amplification was found in four cases (19.0%). p21WAF1 expression was detected in 12 cases (57.1%). MIB-1-LI showed very low levels in all the cases and no significant correlation with p53 or MDM2 immuno-reactivity. None of the tumors showed H-ras mutations. In conclusion, the number of p53 gene alterations in low-grade central osteosarcomas is lower than that in conventional high-grade osteosarcomas. MDM2 alterations and p21WAF1 expression might be involved in the tumorigenesis of low-grade central osteosarcomas.

Pediatr. Res. (18)


http://www.pedresearch.org/cgi/content/abstract/55/3/485

Immature renal tubules are more tolerant to ischemia than mature renal tubules. Here we compared the developmental pattern for some cellular responses evoked by hypoxia and reoxygenation in renal proximal tubules from 10- and 40-day-old rats. Redistribution of Na+-K+-ATPase from the plasma membrane was studied by confocal microscopy techniques in primary cultured renal proximal tubular cells. The developmental expression of Na+-K+-ATPase, {micro}-calpain and heme oxygenase-1 was measured by RT-PCR techniques in rat renal cortex. In response to hypoxia Na+-K+-ATPase redistribution from the plasma membrane was almost 2-fold increased in cells isolated from mature kidneys compared with cells isolated from immature kidneys. Reoxygenation resulted in a complete reestablishment of Na+-K+-ATPase in the plasma membrane in the immature but not in the mature cells. The dissociation of Na+-K+-ATPase from the plasma membrane was associated with a reduced activity and a reduced expression of Na+-K+-ATPase in the mature but not in the immature tubular cells. The expression of {micro}-calpain, a factor shown to induce ischemic injury to proximal tubular cells, was significantly lower in the immature compared with the mature kidney, whereas the expression of heme oxygenase-1, a factor shown to protect from renal ischemic injury, was significantly higher in the immature kidney. The results help to explain the increased tolerance of the immature kidney to injury caused by ischemia and reperfusion.


http://www.pedresearch.org/cgi/content/abstract/53/4/558

In the extremely preterm infant, high transepidermal water loss (TEWL) can result in severe dehydration. TEWL has been attributed to the structural properties of the epidermis but might also be influenced by mechanisms that facilitate water transport. To investigate whether aquaporins (AQP) may be involved in the extreme losses of water through immature skin, we examined the presence and cellular distributions of AQP-1 and AQP-3 in embryonic and adult rat skin by immunohistochemistry. The expression of AQP mRNA in skin was analyzed with the use of semiquantitative reverse transcription-PCR. In rat pups of different embryonic (E) and postnatal
AQP-1 was detected in dermal capillaries, and AQP-3 was abundant in basal epidermal layers. Both AQP displayed several times higher expression in embryonic than in adult skin. TEWL was highest at embryonic day 18 (E18) (133 +/- 1 g/m2h) and lower at E20 (25 +/- 1 g/m2h) and P4 (9 +/- 2 g/m2h). Skin hydration measured as skin electrical capacitance paralleled TEWL, being highest in fetal skin (794 +/- 15 pF at E18) and decreasing to 109 +/- 11 pF at E20 and to 0 +/- 0 pF at P4. We conclude that, as in infants, water loss through the skin of rats decreases markedly with maturation during the perinatal period. The expression and cellular localization of the AQP are such that they might influence skin hydration and water transport and contribute to the high losses of water through the immature skin.


http://www.pedresearch.org/cgi/content/abstract/51/1/106

The C protein {alpha}- and {beta}-antigens are immunodominant components of the surface of Streptococcus agalactiae, the most frequent cause of neonatal sepsis. Both proteins are thought to contribute significantly to virulence of S. agalactiae. They are mainly expressed by serotypes Ia, Ib, and II. The C protein {beta}-antigen (C{beta}-protein) binds to the Fc portion of human IgA and seems to be of importance in bacterial resistance to mucosal immune defense mechanisms. In this study, PCR analysis of S. agalactiae isolates obtained from 189 neonates and 112 pregnant women revealed the presence of the C{beta}-protein gene in 19% and 22% of the isolates, respectively. Size polymorphisms of the PCR products within the gene region encoding the cell wall-spanning domain indicated a high degree of genetic variability. Thirteen different variants of the amplified region were differentiated among the 60 C{beta}-protein-positive isolates by sequence analysis. In all variants, the polymorphisms were caused by insertions and deletions of repetitive DNA elements that did not alter the open reading frame. Comparison of the C{beta}-protein gene polymorphisms showed a significantly higher rate of isolates carrying deletions >50 bp in serotype Ib than in serotype II isolates (p = 0.001); this was also true for neonatal isolates analyzed separately (p = 0.01). Neonatal isolates carried a higher rate of large deletions when compared with maternal isolates; this difference, however, did not reach statistical significance (p = 0.08). We hypothesize that polymorphisms in the cell wall-spanning domain of the C{beta}-protein are of functional relevance with regard to maternofetal transmission of the pathogen.


http://www.pedresearch.org/cgi/content/abstract/55/6/979

Nitric oxide (NO) production may depend on the uptake of L-arginine (L-arg), the substrate for NO synthase in inflammatory lung diseases. The cellular transport of L-arg occurs via the cationic amino acid transporters (CAT), and L-lysine (L-lys) competitively inhibits CAT. Neonatal pigs were treated with lipopolysaccharide (LPS) or vehicle for 4 h. LPS increased exhaled NO (exNO; 0.026 +/- 0.003 to 0.046 +/- 0.003 nmol {middle dot} kg-1 {middle dot} min-1; p < 0.005) and decreased mean systemic arterial blood pressure (89 +/- 4 to 67 +/- 4 mm Hg; p < 0.05), whereas vehicle did not affect exNO or mean systemic arterial blood pressure. The lungs were then isolated and perfused; exNO was greater in lungs from LPS-treated animals (0.08 +/- 0.01 nmol/kg/min) than in lungs from vehicle-treated animals (0.05 +/- 0.01 nmol {middle dot} kg-1 {middle dot} min-1; p < 0.05). The addition of L-arg (0.3 mM) significantly (p < 0.05) increased
exNO production in both groups of lungs (mean increase 0.04 ± 0.01 nmol {middle dot} kg-1 {middle dot} min-1 LPS-treated lungs, p < 0.05; mean increase 0.02 ± 0.01 nmol {middle dot} kg-1 {middle dot} min-1 vehicle-treated lungs); however, L-arg decreased pulmonary vascular resistance (PVR) only in LPS-treated lungs (mean decrease 0.03 ± 0.01 mm Hg {middle dot} ml-1 {middle dot} kg-1 {middle dot} min-1, p < 0.05). L-lys caused a dose-dependent decrease in exNO production and a dose-dependent increase in PVR in LPS-treated lungs. L-lys decreased exNO only at 30 mM and had no effect on PVR in vehicle-treated lungs. In four lungs each from vehicle- and LPS-treated animals, reverse transcriptase-PCR demonstrated CAT-2 mRNA only in LPS-treated animals. These results suggest that the increased NO production in the lungs from LPS-treated animals depends on the uptake of vascular L-arg.


http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000161409.04177.36v1

Thyroid hemiagenesis is a rare form of thyroid dysgenesis of which some familial cases have been reported, including one associated with a heterozygous mutation in the Pax8 gene. However, the physiopathology remains not well known. The objectives of this study were 1) to describe the clinical features, 2) to look for familial clustering, and 3) to search for Pax8 mutations in a relatively large cohort of affected patients. A family history of thyroid dysgenesis was found in nine patients (40%), whose affected relatives had ectopic thyroid (n = 4), athyreosis (n = 1), thyroid hemiagenesis (n = 2), or thyroglossal duct cysts (n = 2). Screening for Pax8 mutations identified abnormal migration profiles by SSCP analysis in 3 patients, but direct sequencing did not show coding region mutations in any of the 22 patients. In conclusion, this study provides the first evidence that thyroid hemiagenesis can occur as a familial disorder associated with any form of thyroid dysgenesis. This finding supports both a common underlying mechanism to the various abnormalities in thyroid development and a role for genetic factors; however, our results from Pax8 analysis suggest that this gene may not be a key factor.


http://www.pedresearch.org/cgi/content/abstract/54/1/26

An alternation of {gamma}-aminobutyric acid (GABA)-ergic neurotransmission has been implicated as an etiologic factor in epileptogenesis. Missense mutations in the GABRG2 gene, which encodes the {gamma}2 subunit of central nervous GABAA receptors, have recently been described in one family with childhood absence epilepsy and febrile seizures (FSs). FSs represent the majority of childhood seizures and have a genetic predisposition. It is not known, however, whether polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FSs. By performing an association study, we used single-nucleotide polymorphisms to investigate the distribution of genotypes of GABRG2 in patients with FSs. A total of 104 children with FSs and 83 normal control subjects were included in the study. PCR was used to identify the C/T and A/G polymorphisms of the GABRG2 gene on chromosome 5q33. Genotypes and allelic frequencies for the GABRG2 gene polymorphisms in both groups were compared. The GABRG2 (nucleotide position 3145 in intron G[-&gt;A]) gene in both groups was not significantly different. In contrast, the number of individuals with the GABRG2 (SNP211037)-C/C genotype in patients with FSs was significantly greater compared with that in healthy control subjects (p = 0.017), and the GABRG2 (SNP211037)-C allele frequency in
patients with FSs was significantly higher than that in healthy control subjects \((p = 0.009)\). The odds ratio for developing FSs in individuals with the GABRG2 (SNP211037)-C/C genotype was 2.56 compared with individuals with the GABRG2 (SNP211037)-T/T genotype. These data suggest that the GABRG2 gene might be one of the susceptibility factors for FSs.


http://www.pedresearch.org/cgi/content/abstract/52/2/155

Platelet-activating factor (PAF) has been implicated in the pathogenesis of gastrointestinal diseases such as necrotizing enterocolitis, Crohn's disease, and ulcerative colitis. However, neither the physiologic role of PAF in the intestine, nor the mechanisms by which PAF participates in the pathogenesis of disease are well understood. The aim of the present study was to determine the direct effect of PAF on intestinal epithelial cell ion transport, and to delineate the mechanisms of regulation. Ion transport was evaluated by measuring short circuit current (Isc) in HT29-CL19A cell monolayers using Ussing chambers. PAF receptor polarity was assessed using domain-selective biotinylation followed by immunoprecipitation and streptavidin blotting of intact epithelial monolayers. PAF (1-200 \(\mu\)M) stimulated Isc that followed the direction of a Cl-gradient and was specifically inhibited by the Cl-channel blockers glybenclamide, 2,2’-iminodibenzoinic acid and 4,4’ diisothiocyanostilbene-2, 2’ disulfonic acid, but was unaffected by the inhibition of prostaglandin synthesis with indomethacin. Stimulated Isc was only detected after apical addition of PAF, correlating with the results of biotinylation experiments indicating an exclusive apical polarity of the PAF receptor. PAF receptor antagonists CV6209 and octylonium bromide abolished PAF-stimulated Isc. Thus, mucosal acting PAF directly and specifically stimulates ion transport via activation of an apical Cl-channel in intestinal epithelial cell monolayers independent of prostaglandin biosynthesis.


http://www.pedresearch.org/cgi/content/abstract/51/4/511

Newborn screening for galactosemia yields a high number of false-positive results. Confirmatory DNA testing for unknown galactosemia mutations on the initial positive sample using novel techniques of mutation detection tenders itself to reduce the recall rate. The potential benefits of confirmatory DNA testing, however, could be offset by the detection of a high percentage of galactosemia carriers, Duarte/galactosemia compound heterozygotes, and infants with benign sequence changes in the galactose-1-phosphate uridytransferase (GALT) gene among infants with a positive biochemical screening test. Our aim was to determine the frequency and allelic distribution of all sequence changes in the GALT gene in 110 newborns with a positive total galactose screening test among 43,688 Austrian newborns screened consecutively. We found that only 20 of the 110 probands carried at least one known or novel candidate galactosemia mutation (one galactosemia homozygote, 7 Duarte/galactosemia compounds, 12 carriers) as judged by denaturing gradient gel electrophoresis and cleavage fragment length polymorphism analysis. Four novel galactosemia candidate mutations (Q9H, A46fsdelCAGCT, M129T, L342I) were identified. Sixty-seven probands had no detectable sequence changes and 23 carried only the benign Duarte or Los Angeles variant alleles or silent mutations. We conclude that a rapid and automatable confirmation test for unknown GALT mutations, e.g. on a high-density oligonucleotide array basis, has the potential to lower the recall rate of galactosemia screening in our population by about five-fold from 0.25 to 0.046%. Further research, however, will be required before the development of such a test can be advocated.

http://www.pedresearch.org/cgi/content/abstract/53/1/18

The isolation of human fetal DNA from the maternal circulation has provided a source of fetal material for prenatal diagnosis. The objective of this study was to investigate whether a similar pattern could be observed in the maternal circulation of male-bearing gravid rhesus monkeys. A real-time PCR TaqMan system for the rhesus Y-chromosome sex determining region was used to determine fetal sex and to quantify fetal DNA concentrations. Results in 14 healthy pregnancies indicated that fetal male DNA could be routinely detected in maternal serum by 50 d of gestation (late first trimester; term 165 +/- 10 d). Fetal DNA concentrations increased with advancing gestation, reaching a mean of 341 genome equivalents/mL of serum (range 11-1570 copies/mL) in the last trimester of gestation, similar to findings in humans. The fetal DNA concentration corresponded to 2.7% of the total maternal serum DNA in the third trimester. Similar to findings in humans, male fetal DNA sequences were not detected postpartum (through 4 wk postpartum) or in animals with a previous history of delivering male offspring. These data indicate that fetal male DNA is present in the maternal circulation of gravid rhesus monkeys comparable to findings in humans and further support the use of this nonhuman primate species as a model to investigate fetomaternal cell trafficking and microchimerism.


http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000156500.13600.B5v1

Neutropenia is a common sequela of neonatal sepsis. Recent clinical trials have shown the beneficial effects of colony-stimulating factors (CSFs) on outcome in this group, but the exact mechanism remains unknown. Neonates and mothers who were at high-risk for infection were recruited for cord blood sampling in a university tertiary referral maternity hospital. Neonatal and adult neutrophils were evaluated for their ability to combat bacterial infection by examining their functional activity (CD11b and reactive oxygen intermediates) and their persistence at inflammatory sites (apoptosis). The mechanism for altered apoptotic responses was assessed by caspase activation assays, X chromosome-linked inhibitor of apoptosis protein expression, and cytoplasmic cytochrome c release. Although granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) significantly delayed neutrophil apoptosis in normal adults, only G-CSF had a similar effect in normal neonates. Neutrophils from neonates who are at high risk for infection are unresponsive to the antiapoptotic effects of G-CSF or GM-CSF, unlike maternal neutrophils, which have delayed apoptosis in response to GM-CSF. However, CD11b expression and reactive oxygen intermediate production were significantly increased in normal neonatal neutrophils that were incubated with GM-CSF versus controls but not G-CSF or lipopolysaccharide. Decreased cytoplasmic cytochrome c release and caspases 3 and 9 activity are associated with the CSF-mediated delay in apoptosis in adults but not in newborns. The antiapoptotic X chromosome-linked inhibitor of apoptosis protein is up-regulated in neonates compared with adults and may mediate their differential spontaneous apoptosis. These results have important implications for the use of CSFs in neonatal sepsis, as responses differ from those seen in adults. Further delineation of neonatal neutrophil responses to CSFs may improve their therapeutic potential.

http://www.pedresearch.org/cgi/content/abstract/56/3/396

Precore nucleotide 1896 and core promoter mutations may account for hepatitis B e antigen (HBeAg) seroconversion in chronic hepatitis B virus (HBV) infection, yet the mutational profiles of the core promoter are largely unknown in children. An age-matched, case-control study enrolled 110 chronic HBV-infected children, including 55 HBeAg seroconverters and 55 nonseroconverters. Precore and core promoter genes of HBV were sequenced and the serum viral genomes were genotyped from three serial serum samples of the seroconverters and from one serum sample of the nonseroconverters. Higher frequency of A1775G and G1799C mutation rates and lower frequency of A1752G mutation rate were found in the seroconverters. Precore 1896 mutation appeared more in seroconverters than in nonseroconverters (45.5% versus 10.9%; p < 0.001). 1762 + 1764 mutation rates were not different between the seroconverters (9.1%) and the nonseroconverters (5.5%). Genotype B was the major type. Genotype C was associated with core promoter 1762 + 1764 mutations in the seroconverter group (p = 0.023). The conclusions of this study include the following: 1) mutations of core promoter at nucleotide position 1752, 1775, and 1799 have significant correlations with HBeAg seroconversion; 2) core promoter 1762 + 1764 mutations play a minimal role in HBeAg seroconversion; 3) precore 1896 mutant accounted for half of childhood HBeAg seroconversion; 4) genotype C is associated with 1762 + 1764 mutations during the process of HBeAg seroconversion.


http://www.pedresearch.org/cgi/content/abstract/55/4/657

Neurogenic inflammation is markedly potentiated in airways that are infected with respiratory syncytial virus (RSV). Aims of this study were to determine whether this potentiation persists after the virus is cleared, investigate the mechanism of postviral potentiation, and define whether prophylaxis with a MAb against the RSV fusion protein (palivizumab) prevents this effect. Thirty days after inoculation, no evidence of active RSV infection was found in the airway epithelium by plaque assay or immunostaining and no viral nucleic sequences were detected by PCR, yet capsaicin-induced plasma extravasation in the airways that were infected 30 d earlier with RSV was still significantly larger compared with pathogen-free controls. Substance P content in lung tissues and capsaicin-induced release of this peptide from sensory nerves were significantly increased at 30 d. The administration of palivizumab 24 h before virus inoculation prevented the development of abnormal neurogenic inflammatory responses. Our data suggest that the airways remain abnormally susceptible to the proinflammatory effects of sensory nerves after RSV infection is cleared, as a result of changes in sensory innervation, and that this abnormality can be prevented by passive prophylaxis against RSV.


http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000157674.63621.2Cv1

Nail-patella syndrome (NPS) is an autosomal dominant disease characterized by dysplastic nails, absent or hypoplastic patellae, elbow dysplasia, and nephropathy. Recently, it was shown that NPS is the result of heterozygous mutations in the LIM-homeodomain gene, LMX1B.
Subsequently, many mutations of the LMX1B gene have been reported in NPS patients. However, functional analyses of the mutant proteins have been performed in only a few mutations. Furthermore, the mechanisms of dominant inheritance in humans have not been established. In the present study, we analyzed the LMX1B gene in three Japanese patients with NPS and identified two novel mutations, 6 nucleotide deletion (Δ246N 247Q) and V242L. These two mutations are located in the homeodomain of LMX1B. Functional analyses of the LMX1B mutants revealed that these mutants had diminished transcriptional activity and had lost DNA binding ability. Furthermore, we demonstrated that each mutant did not manifest a dominant-negative effect on the transcriptional activity of wild-type LMX1B. These results suggested that NPS is caused by loss-of-function mutations of LMX1B, and haploinsufficiency of LMX1B should be the predominant pathogenesis of NPS in humans.


http://www.pedresearch.org/cgi/content/abstract/55/1/147

The present study examines the hypothesis that morphine exposure alters newborn brain vascular endothelial cell production of endothelin (ET)-1, as well as the mRNA expression of its receptors. Newborn piglet vascular endothelial cells were treated with morphine (100 ng/mL media), naloxone (100 ng/mL media), or drug-free media (control) for 6, 24, 48, and 96 h. Media was analyzed for ET-1 and big ET-1 levels and the cells were assessed for ETA and ETB receptor mRNA expression. Morphine exposure progressively increased ET-1 production from 6 to 96 h with concurrent reductions in big ET-1 levels starting at 24 h to almost undetectable levels by 96 h. Whereas ETA receptor mRNA expression increased 2-fold at 6 h and 4-fold at 96 h, ETB receptor mRNA expression remained unchanged. Naloxone exposure caused significant decreases in ET-1 levels, whereas an opposite effect was noted in big ET-1 levels, which increased from 6 through 96 h. Naloxone caused a progressive decrease in ETA receptor mRNA expression at 6 h through 96 h and a 2-fold increase in ETB receptor mRNA expression at 48 and 96 h. Increased ET-1 and its receptors in response to morphine may suggest altered cerebrovascular perfusion and brain metabolism in the immature piglet brain.


http://www.pedresearch.org/cgi/content/abstract/52/6/879

There is evidence that fetal growth restriction is associated with impaired nephrogenesis and reduced numbers of mature nephrons at birth. It has been proposed that such impairment of renal growth may contribute to increased blood pressure in later life. Although prostaglandins (PG) play a key role in kidney development, it is unknown whether a poor fetal substrate supply alters the synthesis or actions of PG within the fetal kidney. Using real-time reverse transcriptase PCR, we have measured the effect of chronic placental restriction (PR) on the renal expression of PG endoperoxide G/H synthase-2 (PGHS-2), PGE2 receptors EP2 and EP4, and renin mRNA in the sheep fetus in late gestation. Restriction of placental growth reduced fetal body weight (PR: 3.2 {+/-} 0.2 kg, control: 4.8 {+/-} 0.2 kg) and total kidney weight (PR: 19.7 {+/-} 1.8 g, control: 25.1 {+/-} 1.3 g). Mean fetal arterial PO2 was reduced by PR (PR: 15.03 {+/-} 0.67 mm Hg, control: 21.3 {+/-} 0.87 mm Hg). Renal PGHS-2 mRNA was increased in the PR group (PR: 2.26 {+/-} 0.38, control: 1.20 {+/-} 0.31) and was inversely related to mean fetal arterial PO2 in the PR and control groups [PGHS-2: -0.17 (PO2) + 4.69, r2 = 0.26]. PR also increased renal EP2 (PR: 1.57 +
Renal prostaglandins (PG), renin, and cortisol are necessary for normal kidney development and function during fetal life. We examined the effects of cortisol infusion before completion of nephrogenesis (d 109-116 gestation; 2.0-3.0 mg hydrocortisone succinate/24 h) on the renal mRNA expression of PGHS-2, the PGE2 receptors, EP2 and EP4, and renin in fetal sheep. Cortisol infusion raised plasma cortisol levels to 42.8 +/- 6.0 nmol/L compared with saline infusion levels of 1.5 +/- 0.5 nmol/L (p < 0.001), but had no effect on fetal body weight, proportional kidney mass, or blood gases. Cortisol decreased significantly the relative expression of renin mRNA (saline: 0.93 +/- 0.06 units; cortisol: 0.32 +/- 0.03 units, p < 0.05), however it had no effect upon the expression of PGHS-2, EP2, or EP4 mRNA in fetal sheep kidney. Although there is substantial evidence that PGE2 acting through either the EP2 or EP4 receptor stimulates renin synthesis in the adult kidney, our results have demonstrated that before the completion of nephrogenesis, cortisol down-regulation of renin mRNA expression is independent of any change in the expression of PGHS-2, EP2, or EP4 mRNA expression. During nephrogenesis, the insensitivity of PGHS-2, EP2, and EP4 expression to down-regulation by cortisol may permit continued PG regulation of renal development and urine formation.


http://www.pedresearch.org/cgi/content/abstract/54/6/819

Apolipoprotein E (APOE) regulates cholesterol and fatty acid metabolism, and may mediate synaptogenesis during neurodevelopment. To our knowledge, the effects of APOE4 isoforms on infant development have not been studied. This study was nested within a cohort of mother-infant pairs living in and around Mexico City. A multiple linear regression model was constructed using the 24-mo Mental Development Index (MDI) of the Bayley Scale as the primary outcome and infant APOE genotype as the primary risk factor of interest. Regression models stratified on APOE genotype were constructed to explore effect modification. Of 311 subjects, 53 (17%) carried at least one copy of the APOE4 allele. Mean (SD) MDI scores among carriers with at least one copy of APOE4 were 94.1 (14.3) and among E3/E2 carriers were 91.2 (14.0). After adjustment for covariates, APOE4 carrier status was associated with a 4.4 point (95% confidence interval: 0.1-8.7; p = 0.04) higher 24-mo MDI. In the stratified regression models, the negative effects for umbilical cord blood lead level on 24-mo MDI score was approximately 4-fold greater among APOE3/APOE2 carriers than among APOE4 carriers. These results suggest that subjects with the E4 isofom of APOE may have advantages over those with the E2 or E3 isoforms with respect to early life neuronal/brain development.
The stage of maturation of monocytes affects their susceptibility to HIV infection. The \(\beta\)-chemokines and their receptor CCR5 play a crucial role in inflammatory reactions and HIV infection. We therefore examined the correlation between the expression of CCR5 and \(\beta\)-chemokine production and the susceptibility to HIV infection during cord monocyte (CM) differentiation into macrophages. CM and CM-derived macrophages (CMDM) were examined for \(\beta\)-chemokine and CCR5 expression. The susceptibility of the CM cultured in vitro at different time points to HIV infection was also determined. Although the levels of CCR5 mRNA expression in freshly isolated CM are comparable to those in CMDM, CM had significantly lower levels of CCR5 protein on the cell surface than CMDM did. Steady increase of CCR5 protein expression on the cell surface was observed during CM differentiation into macrophages. The CCR5 expression correlated with the increased susceptibility to HIV infection by CMDM. Although there was no significant difference in endogenous \(\beta\)-chemokine production between CM and CMDM, HIV infection of CMDM significantly enhanced production of macrophage inflammatory protein-1\(\alpha\) and -1\(\beta\). CCR5 receptor plays a critical role in HIV infection of neonatal blood monocyte/macrophages.
We report a 20-year-old man with gigantism syndrome, hypertrophic cardiomyopathy, muscle weakness, exercise intolerance, and severe psychomotor retardation since childhood. Histochemical and biochemical analysis of skeletal muscle biopsy revealed myoadenylate deaminase deficiency; molecular genetic analysis confirmed the diagnosis of primary (inherited) myoadenylate deaminase deficiency. Plasma, urine, and muscle carnitine concentrations were reduced. -Carnitine treatment led to gradual improvement in exercise tolerance and cognitive performance; plasma and tissue carnitine levels returned to normal, and echocardiographic evidence of left ventricular hypertrophy disappeared. The combination of inherited myoadenylate deaminase deficiency, gigantism syndrome and carnitine deficiency has not previously been described.


Two patients are described in a family with a mitochondrial DNA T8993C point mutation. Patient 1, the proband, was a 4-year-old male, and his clinical features were consistent with those of Leigh syndrome, including lactic acidosis, motor development delay, and symmetric basal ganglia lesions on magnetic resonance imaging (MRI). His mental development was delayed mildly, but he has not demonstrated neurologic deterioration. Patient 2 was his maternal aunt. She developed her first neurologic sign at 18 months of age, thereafter her development ceased and regressed. She had lost her head control and become bedridden by 4 years of age and died at 20 years of age, demonstrating a more severe clinical course than that of Patient 1. Analysis of mitochondrial DNA from peripheral leukocytes of Patient 1 revealed a T8993C mutation of 99%. Patient 2 was demonstrated to have the same mutation at high abundance (99%) in the frozen myocardium and in the formaldehyde preserved spinal cord, with only 18% mutant mitochondrial DNA present in the formaldehyde preserved sciatic nerve. The mother of Patient 1, who was phenotypically normal (sister of Patient 2), had 35% mutant mitochondrial DNA in peripheral leukocytes. The authors' findings suggest that T8993C phenotypes are highly variable and that the proportion of the mutant mitochondrial DNA may vary among tissues and not correlate well with clinical severity.


The frequency was studied with which human herpesviruses types 6 and 7 (HHV-6 and HHV-7) occur in the cerebrospinal fluid (CSF) of patients with febrile seizures and matched control patients. CSF samples were prospectively collected from a case series of patients with febrile seizures and from age-, sex-, and race-matched control patients without febrile seizures, all of whom were evaluated in the emergency department of an urban, tertiary care, pediatric medical center. Using polymerase chain reaction, the samples were examined for the presence of viral DNA from HHV-6, HHV-7, herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), and cytomegalovirus (CMV). CSF from a subset of both groups was also examined for RNA from
enteroviruses. During the 7-month, 2-week collection period, a total of 174 patients were evaluated for fever and seizures. Of these, 23 (13.2%) met the study criteria. Their mean age was 1.4 +/- 0.7 years. Sixteen (70%) of the 23 were male. The 23 patients were matched to 21 control subjects. None of the samples from the patients or control subjects had polymerase chain reaction evidence of HHV-6, HHV-7, HSV-1, or HSV-2. All samples from the patients were negative for CMV. One control subject was positive for CMV. The 10 patients and seven control subjects tested for enteroviral RNA were negative. Neither HHV-6 nor HHV-7 appears to be present in the CSF of patients with febrile seizures. What role, if any, they have in the pathogenesis of febrile seizures merits further study.


http://www.sciencedirect.com/science/article/B6TBD-475WPXC-4/2/2ea5e0618eeefe7aed2fc502455b8382

Interleukin-4 (IL-4) is a cytokine with anti-inflammatory properties. This study was undertaken to investigate whether IL-4 intron 3 gene polymorphism could be used as markers of susceptibility to febrile seizures and epilepsy of children. Children were divided into three groups: group 1, febrile seizures (n = 51); group 2, epilepsy (n = 43); and group 3, normal control group (n = 83). Polymorphisms for IL-4 intron 3 were detected by polymerase chain reaction. Genotypes and allelic frequencies for IL-4 intron 3 gene polymorphism in three groups were compared. We found that proportions of different IL-4 intron 3 polymorphisms in three groups were nonsignificantly different. Proportions of RP1 homozygote/heterozygote/RP2 homozygote for IL-4 intron 3 in three groups were as follows: group 1, 56.9/41.2/1.9%; group 2, 62.8/32.6/4.6%; and group 3, 62.7/33.7/3.6%. The proportion of RP1/RP2 for IL-4 intron 3 in three groups were as follows: group 1, 77.5/22.5%, group 2, 79.1/20.9%, and group 3, 79.5/20.5%. We concluded that the association of IL-4 polymorphisms with febrile seizures and epilepsy of children does not exist. IL-4 intron 3 polymorphism is not a useful marker for prediction of the susceptibility of febrile seizure and epilepsy of children.


http://www.sciencedirect.com/science/article/B6TBD-3SJMWJ6-4/2/9a4855a9052a8e0470d5541bf8783a33

In the United States, juvenile neuronal ceroid-lipofuscinosis (JNCL) is the most common form of NCL. This study analyzed 191 cases, diagnosed on the basis of age-at-onset, clinical symptomatology, and pathologic findings. Twenty percent (40/191) of these cases from 24/120 families manifested atypical clinical symptomatology and/or pathologic findings (typical revealed fingerprints and atypical revealed mixed inclusions, or only curvilinear or granular profiles) and, therefore, represent variant forms of JNCL. Those patients in the study with typical JNCL were a uniform group of cases, whereas the atypical were heterogenous and were divided into 8 subgroups based on the clinicopathologic findings. Forty-three families were analyzed (27 typical, 16 atypical) for the common 1.02 kb deletion and several pedigrees for novel mutations. In typical JNCL the common 1.02 kb deletion in both alleles (homozygous) were observed in 23/27, and only 1 allele (heterozygous) was exhibited in 4/27 families. In atypical JNCL families, 5/16 were heterozygous for the common 1.02 kb deletion. None of the remaining 11/16 families had the common 1.02 kb deletion in either allele, but in 9/11 cases the palmitoyl-protein thioesterase (PPT) levels were deficient. In cases where the mutation in CLN3 gene has not been identified, several possibilities may exist. The phenotype may be caused by a yet undefined mutation in
CLN3 or may be due to overlapping with other forms of NCL.

**Objective.** Although rhinitis is extremely frequent in children, methods for assessing the severity of nasal inflammation produce results with wide variability and hence weak clinical significance. We designed this epidemiologic investigation to define the clinical usefulness of assessing nasal cellularity in children. Methods. We studied 183 of 203 eligible unselected schoolchildren who were aged 9 to 11 years and whose parents gave informed consent and completed a questionnaire on the history of atopic and respiratory symptoms. In all children, nasal swabs were obtained from both nostrils and eluted in saline and slides were prepared from cytospin preparations for staining and white cell counts. Children also underwent determination of nasal volume, skin prick tests with 7 common local allergens, flow volume curves, and nitric oxide measurement in expired air. Blood samples were drawn for the measurement of total immunoglobulin E, eosinophil percentage, and detection of Chlamydia pneumoniae antibodies. C pneumoniae DNA was also sought in eluates from nasal swabs. The percentage, standard deviations, and percentiles of the various nasal white cell populations were determined. Results. No correlation of the percentage of these cells was found with the history of allergies or respiratory disease or with functional or laboratory finding. Repeat nasal swabs obtained 1 month after the initial examination in 31 children (20 with neutrophils higher and 11 lower than 14%) in 77.4% of the cases confirmed the previous (high or normal) result. Twelve of the 16 eligible children with persistently high nasal neutrophil counts completed a 15-day cycle of intranasal flunisolide therapy (200 {micro}g twice a day). Therapy significantly reduced nasal neutrophil percentage and increased nasal volume. Conclusions. Increased nasal neutrophils, although related neither to the clinical history nor to laboratory variables, are a common important finding in children. A 15-day cycle of intranasal flunisolide is sufficient to restore normal nasal neutrophilia.

Background. Delayed growth is a well-established feature of pediatric Crohn's disease. Several factors have been shown to affect growth, including disease location, severity, and treatment. The recently discovered NOD2 gene has been correlated to ileal location of Crohn's disease and subsequently could affect growth through the resulting phenotype or as an independent risk factor. The aim of our study was to determine if growth retardation is affected by genotype independently of disease location or severity. Methods. Genotyping for 3 NOD2 single-nucleotide polymorphisms was performed in 93 patients with detailed growth records. Parameters including disease location, disease severity, and NOD 2 genotype and their effect on z scores for height and weight at disease onset and during follow-up were analyzed. Results. NOD2 mutations were correlated with ileal location but not with disease severity or growth retardation. Ileal involvement
was significantly associated with height retardation at disease onset and the lowest z score during follow-up. Use of steroids affected weight but not height. Regression models for growth variables revealed that the strongest association with impaired growth is with disease severity (weight- and height-failure odds ratios: 6.17 and 4.52, respectively). Conclusions. Severity of disease is correlated with growth failure for both height and weight. Location of disease is a weaker predictor of disordered growth and is correlated with growth retardation but not growth failure. The NOD2 genotype was not correlated with growth retardation or growth failure.


http://pediatrics.aappublications.org/cgi/content/abstract/109/5/826

Objective. To evaluate the effect of uncomplicated viral respiratory infections (colds) on middle ear pressure in healthy school-aged children. Methods. Children (ages 2-12) with normal tympanograms before onset of illness had bilateral tympanometry daily except weekends for 2 weeks after the onset of a cold. Nasopharyngeal secretion obtained at onset of illness was cultured for bacterial pathogens of otitis media using selective agars and tested for rhinovirus, coronavirus, respiratory syncytial virus, influenza A and B, and parainfluenza 1-3 by reverse transcriptase polymerase chain reaction technology. Tympanometry was designated as abnormal with peak pressure of $\leq -100$ daPa or $\geq 50$ daPa and/or a compliance peak of $<0.2$ cm3. Results. Eighty-six colds were studied, 82 in schoolchildren (5-12 years old) and 4 in 2- to 3-year-olds. Abnormal negative middle ear pressure occurred at least once during the 2 weeks after onset in 57 (66%) of the 86 colds. Tympanometry was abnormal in the first week after onset in 50 (88%) of the 57 colds and was abnormal on a single day in 17 (30%) of the 57. The middle ear pressure abnormalities were intermittent and shifted from one ear to the other ear from day to day. Reverse transcriptase polymerase chain reaction was positive for a respiratory virus in 56 (65%) of the 86 illnesses. Rhinovirus was found in 48% and respiratory syncytial virus in 14%. Pathogenic bacteria (Streptococcus pneumoniae, Haemophilus influenzae, or Moraxella catarrhalis) were detected in nasopharyngeal secretion in 29 (34%) of the 86 colds; the bacteria were in high titer ($\geq 10^3$ cfu/mL) in 26 of the 29 positive specimens. None developed illness that required a visit to a physician. Age, detection of a respiratory virus, and presence of bacterial pathogen in the nasopharyngeal secretion had a negligible effect on the occurrence of abnormal tympanometry. Occurrence of negative middle ear pressure in winter-spring colds was significantly greater than in fall colds for unexplained reasons. Conclusions. Transient negative middle ear pressure occurred in two thirds of uncomplicated colds in healthy children. This negative pressure, which may facilitate secondary viral or bacterial otitis media, seems to result from viral infection of the nasopharynx and distal tube causing bilateral eustachian tube dysfunction. Tympanometry provides an objective measure of the potential beneficial effects of investigational treatments on the risk of eustachian tube dysfunction/otitis media.


http://pediatrics.aappublications.org/cgi/content/abstract/111/6/1333

Objective. Preterm infants are at greater risk of symptomatic cytomegalovirus (CMV) infection than term infants. Breast milk is the main source of perinatal CMV infections. This study evaluated the kinetics of CMV load in breast milk and the rate of postnatal CMV transmission via breast milk from mothers to their preterm infants. Methods. This was a prospective study of 30 mothers and their 43 preterm infants. The infants either had a gestational age of $<34$ weeks or
weighed <2000 g at birth. Breast milk, serum, and urine samples were collected every 2 weeks until discharge, and screened for CMV infection using a real-time PCR assay. Most of the breast milk had been preserved at -20\(\degree\)C before feeding to the preterm infants. Results. Twenty-four mothers (24 of 30, 80%), who had 34 preterm infants, were CMV immunoglobulin G positive. Twenty-one (87.5%) of the 24 seropositive mothers, who had 30 preterm infants, had detectable CMV deoxyribonucleic acid (DNA) in breast milk during the study period. Most breast milk became positive for CMV DNA 2 weeks after delivery. Viral DNA copy numbers increased until they peaked at 4 to 6 weeks. Afterward, the CMV DNA copy numbers decreased. Of the 30 infants who were fed CMV DNA-positive breast milk, CMV infection was confirmed in 3 infants. However, they had no clinical symptoms of CMV infection. Conclusions. Despite the high rate of CMV DNA in breast milk, symptomatic infections in the preterm infants did not occur. These results might be associated with the method of breast milk preservation and the population we studied. CMV infections transmitted via breast milk feeding did not have much impact on preterm infants in our institutes.

Peptides (22)


http://www.sciencedirect.com/science/article/B6T0M-4DTKP91-1/2/ce5083202180cd8eb26fe8a6d7252a2

Previous studies performed in this laboratory have demonstrated that the fetal lung contains immunoreactive adrenocorticotropin (irACTH), and that the lung both clears and secretes irACTH under basal and stimulated conditions. Furthermore, we have demonstrated that the irACTH in fetal lung is accounted for by proopiomelanocortin (POMC), and that there is an evidence of post-translational processing that is distinct from the pattern of processing typical of the anterior pituitary. The present study was designed to test the hypothesis that POMC is synthesized in the fetal lung, and that there is decreased synthesis in the late-gestation ovine fetal lung. Lungs were collected from fetal sheep at 80, 100, 120, 130, and 145 days gestation (n = 4/group; term = 147 days). POMC mRNA was measured using reverse transcription and real-time polymerase chain reaction with probe and primers designed in this laboratory. The greatest abundance of POMC mRNA was in the 80-days fetal sheep, and the relative abundance decreased as a function of fetal gestational age. POMC protein was measured using immunoblot analysis in lungs from 80, 120, and 145-days fetal sheep. The pattern of POMC protein abundance was consistent with that of the mRNA (highest at 80 days, lowest at 145 days). The POMC immunoblot revealed specific staining of a peptide with molecular weight of 27 kDa and another peptide with a molecular weight slightly higher than that of native POMC (32 kDa). For comparison, we measured POMC mRNA in skeletal muscle and small intestine. We found POMC expression in both fetal tissues, but no statistically significant ontogenetic pattern of expression. We conclude that POMC is synthesized in the ovine fetal lung, and that the rate of synthesis decreases as the fetus matures in utero. We speculate that the decreasing abundance of POMC mRNA and protein reflects decreased release of POMC and POMC-related peptides into the fetal bloodstream.

We present the molecular cloning and characterization of the human galanin receptor, hGALR2. hGALR2 shares 85%, 39%, and 57% amino acid identities to rGALR2, hGALR1, and hGALR3, respectively. hGALR2, along with rGALR2, can be distinguished from the other cloned galanin receptors by a tolerance for both N-terminal extension and C-terminal deletion of galanin, as well as by a primary signaling mechanism involving phosphatidyl inositol hydrolysis and calcium mobilization. By RT-PCR, GALR2 mRNA was abundant in human hippocampus, hypothalamus, heart, kidney, liver, and small intestine. A weak GALR2 mRNA signal was detected in human retina, and no signal was detected in cerebral cortex, lung, spleen, stomach, or pituitary.


Receptors for calcitonin gene-related peptide (CGRP), a neuropeptide known to be the most potent vasodilator, are abundantly expressed in cerebellum. A monoclonal antibody to cerebellar CGRP receptors specifically detects a 66 kDa protein from rat cerebellum and other rat and human tissues, but not from SK-N-MC cells which express calcitonin receptor-like receptor (CRLR), a recently described component of CGRP receptors. In contrast, mRNA expression for CRLR was abundant in SK-N-MC cells, but it was undetectable in rat cerebellum. Furthermore, the antibody could not detect any immunoreactive protein in HEK 293 cells transiently transfected with CRLR and receptor activity-modifying protein 1 (RAMP1) indicating the possible existence of another CGRP receptor, which does not involve CRLR. Due to the absence of biochemical or structural data on the existence of a CGRP2 receptor and the new data provided in this paper, we suggest to identify the two CGRP receptors as CGRP-A and CGRP-B.


Central dopaminergic systems are implicated in schizophrenia and Parkinson's disease, and are known to be modulated by the endogenous tripeptide Pro-Leu-Gly-NH2 (PLG or MIF-1, melanocyte-stimulating hormone release inhibiting factor-1). Differential display polymerase chain reaction (ddPCR) was utilized to identify genes that are regulated by protracted PLG treatment (20 mg/kg, i.p. for 28 days) in male Sprague-Dawley rats. A total of 2400 genes were screened and 3 down-regulated bands were identified in the PLG-treated samples. Sequencing analysis revealed a total of six unique cDNA species. One fragment possessed a high degree of homology with Mus musculus hnRNP-L (protein L) mRNA (GenBank #AB009392) (termed PRG1: PLG regulated gene 1). Elongation of the PRG1 cDNA, by RACE-PCR, provided an 835 bp sequence with 95% homology to AB009392 over a 743 bp span. Open reading frame analysis provided a putative amino acid sequence consistent with the identity of PRG1 as rat hnRNP-L. Northern hybridization experiments with PRG1 cDNA, by Western blot analysis revealed significantly decreased hnRNP-L levels in the striatum and pre-frontal cortex (but not the nucleus accumbens) by 71 and 61%, respectively of PLG-treated animals. The identification of altered expression of hnRNP-L following
PLG treatment provides insight into the long-term effects of PLG and may provide insight into its molecular mechanism of action.


http://www.sciencedirect.com/science/article/B6T0M-4F7H5FX-1/2/9c003ce8942903b6edbee2fleea400

The rat orphan receptor UHR-1 and its human orthologue, GPR10, were first isolated in 1995. The ligand for this receptor, prolactin-releasing peptide (PrRP), was identified in 1998 by reverse pharmacology and has subsequently been implicated in a number of physiological processes. As supported by its localization and regulation in the hypothalamus and brainstem, we have shown previously that PrRP is involved in energy homeostasis. Here we describe a naturally occurring polymorphism in the UHR-1 gene that results in an ATG to ATA change at the putative translational initiation site. The presence of the polymorphism abolished the binding of 125I PrRP in rat brain slices but did not affect the ability of PrRP to reduce fast-induced food intake. Together this data suggest that PrRP may be exerting its feeding effects through a receptor other than UHR-1.


http://www.sciencedirect.com/science/article/B6T0M-3VYTDF7-K/2/570e1518649e3db3cd26422d5bbc8cf6

Studies using fetal sheep, goats, and guinea pigs indicate that vasopressin may play a role in preparing the fetal lung for the transition from a uterine to an air-breathing environment by slowing lung liquid secretion. The mechanism of vasopressin action is believed to occur through V2 receptors with subsequent activation of amiloride-sensitive sodium channels. However, the presence of the V2 receptor in human lung has not yet been documented. In the present study, expression of the vasopressin V2 receptor in fetal and adult human lung was examined using reverse transcription-polymerase chain reaction (RT-PCR), Northern blot analysis, and DNA sequencing. Using RT-PCR and primer pairs specific for the human V2 receptor, PCR products of the predicted sizes of 512 and 862 bp were obtained from adult human lung. DNA sequencing of the cloned PCR products revealed exact identity with the published sequence for the V2 receptor. Northern blot analysis revealed the expression of a ~ 1.9 kb mRNA in adult human lung as well as in kidney, but not in fetal human lung at 22-24 weeks of gestation. However, using the more sensitive RT-PCR assay the 862-bp product was successfully amplified from human fetal lung, although the data indicate the mRNA for this receptor is expressed in lower levels than in adult human lung or kidney. Using RT-PCR and primers specific for the rat V2 receptor, a PCR product of the predicted size of 461 bp was amplified from adult rat lung and kidney, despite an earlier report that this receptor mRNA is absent from the lung of this species. The role for the V2 receptor in adult human lung is unknown at this time, but, as in the human kidney and lungs of fetal sheep, goats, and guinea pigs, this receptor may play a role in fluid balance.

Guerrero, F. D. Transcriptional expression of a putative tachykinin-like peptide receptor gene from stable fly. Peptides 18(1) 1-5, 1997.--STKR is a 4118 bp clone from a stable fly, Stomoxys calcitrans, cDNA library which encodes a protein with significant amino acid identity to tachykinin-like peptide receptors. Ribonuclease protection assays and RT-PCR were utilized to examine the transcriptional expression of STKR from various life stages of the stable fly. STKR expression was detectable in all stages, but was most abundant in isolated adult fly gut and lowest in developing embryos.


Calcitonin gene-related peptide and adrenomedullin exert potent effects in skin but their cellular targets are unknown. This study aimed to identify the cellular location of calcitonin receptor-like receptor (CRLR) which is pharmacologically identical to CGRP receptor-1, a putative molecular target of CGRP and adrenomedullin. RT-PCR analysis of human hairy skin revealed the presence of CRLR mRNA and immunohistochemical analysis, employing a previously characterized polyclonal antibody raised to CRLR, provided novel evidence of the cellular distribution of CRLR. Extensive and specific CRLR-immunostaining was detected in arteriolar smooth muscle and venular endothelium and is consistent with CGRP's putative role in neurogenic inflammation. Novel targets for CGRP and/or adrenomedullin were identified, including capillary endothelium, hair follicles and sweat glands.


Distribution of adrenomedullin (AM)-containing perivascular nerve fibers was studied in rat mesenteric arteries. Many fibers containing AM-like immunoreactivity (LI) were observed in the adventitia. AM-LI fibers were abolished by cold storage denervation or capsaicin but not 6-hydroxydopamine. Double immunostainings showed colocalization of AM-LI with calcitonin gene-related peptide (CGRP)-LI. The dorsal root ganglia had many AM-positive cells and AM mRNA detected by RT-PCR. Electron microscopy study revealed high proportions of immunogold labeling for AM and colocalization of both AM-LI and CGRP-LI in unmyelinated nerve axons. These results suggest that AM-containing perivascular nerves are distributed in the rat mesenteric artery.

The lack of specific pharmacological tools has impeded the evaluation of the role of each melanocortin receptor (MCR) subtype in the myriad physiological effects of melanocortins. 154N-5 is an octapeptide (MFRdWFKV-PV-NH2) that was first identified as an MC1R antagonist in Xenopus melanophores [J. Biol. Chem. 269 (1994) 29846]. In this manuscript, we show that 154N-5 is a specific agonist for human and murine MC1R. The peptide has negligible activity at MC3R and MC4R and is 25-fold less potent and a weak agonist at MC5R. 154N-5 was tested in both a cellular and an animal model of tumor necrosis factor-[alpha] (TNF-[alpha]) secretion. The inhibitory efficacy of 154N-5 on TNF-[alpha] secretion in both models was similar to the nonselective agonist NDP-[alpha]-melanocyte stimulating hormone (NDP-[alpha]MSH), thus, we conclude that inhibition of TNF-[alpha] secretion by melanocortin peptides is mediated by MC1R. 154N-5 is a valuable new tool for the evaluation of specific contribution of MC1R agonism to physiological and pathological processes.


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LUCAS, G.A., L.R. WHITE, R. JUUL, J. CAPPELEN, J. AASLY AND L. EDVINSSON. Relaxation of human temporal artery by endothelin ETB receptors. PEPTIDES 17(7) 1139-1144, 1996.-- Endothelin receptors have been characterized in human temporal artery by molecular biological methods and in vitro pharmacology. Reverse transcriptase-polymerase chain reaction was used to detect mRNA encoding ETA and ETB receptors in normal and endothelium-denuded arteries. Vasomotor response experiments with a specific ETA antagonist (FR 139317) suggested the presence of ETA subtypes. Marked ETB-mediated relaxation was obtained with ET-3 when ETA activity was blocked in precontracted arteries. Relaxation was significantly reduced by bosentan, indomethacin, and a nitric oxide synthase inhibitor. It may be speculated that the relaxant activity is mediated through ETB1 receptors. Copyright (c) 1996 Elsevier Science Inc.


http://www.sciencedirect.com/science/article/B6T0M-42NY293-9/2/dbeff0d509b12c589263ad44a293ca20

The Y5 receptor has been postulated to be the main receptor mediating NPY-induced food intake in rats, based on its pharmacological profile and mRNA distribution. To further characterize this important receptor subtype, we isolated the Y5 gene in the guinea pig, a widely used laboratory animal in which all other known NPY receptors (Y1, Y2, Y4, y6) [2, 13, 33 and 37] have recently been cloned by our group. Our results show that the Y5 receptor is well conserved between species; guinea pig Y5 displays 96% overall amino acid sequence identity to human Y5, the highest identity reported for any non-primate NPY receptor orthologue, regardless of subtype. Thirteen of the twenty substitutions occur in the large third cytoplasmic loop. The identities between the guinea pig Y5 receptor and the dog, rat, and mouse Y5 receptors are 93%, 89%, and 89% respectively. When transiently expressed in EBNA cells, the guinea pig Y5 receptor showed a high binding affinity to iodinated porcine PYY with a dissociation constant of 0.41 nM. Competition experiments showed that the rank order of potency for NPY-analogues was PYY = NPY = NPY2-36 > gpPP > rPP >> NPY 22-36. Thus the pharmacological profile of the guinea pig Y5 receptor agrees well with that reported for the Y5 receptor from other cloned species.
Before parturition the uterine cervix undergoes a ripening process ("softens" and dilates) to allow passage of the fetus at term. The exact mechanism(s) responsible for cervical ripening are unknown, though a role for peptidergic sensory neurons is emerging. Previous work demonstrated that administration of substance P (SP) to ovariectomized rats caused events associated with cervical ripening, that production of SP in cervix-related dorsal root ganglion (DRG) is estrogen responsive, and that release of SP from neurons terminating in the cervix and spinal cord peaks prior to parturition. The present study was designed to test the hypothesis that calcitonin gene-related peptide (CGRP), a neuropeptide co-stored with SP in many sensory neurons, undergoes changes with pregnancy and hormonal environment. Immunohistochemistry, in situ hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and radioimmunoassay (RIA) were used to investigate CGRP in L6-S1 DRG, spinal cord and cervix during pregnancy and the role of estrogen in CGRP synthesis. CGRP-immunoreactive primary sensory neurons expressed estrogen receptors (ER-[alpha] and ER-[beta]). In the cervix, CGRP concentrations decreased, but in the L6-S1 DRG and the spinal cord segments, CGRP levels increased, with peak effects observed at day 20 of gestation. CGRP mRNA synthesis increased in DRG over pregnancy. Sensory neurons of ovariectomized rats treated with estrogen showed increased CGRP mRNA synthesis in a dose-related manner, an effect blocked by the ER antagonist ICI 182 780. From these results, we postulate that synthesis of CGRP in L6-S1 DRG and utilization in the cervix increase over pregnancy and this synthesis is the under influence of the estrogen-ER system. Collectively, these data are consistent with the hypothesis that CGRP plays a role in cervical ripening and, consequently in the birth process.

Prior to parturition the non-pliable uterine cervix undergoes a ripening process ("softens" and dilates) to allow a timely passage of the fetus at term. The exact mechanism(s) triggering and involved in cervical ripening are unknown, though evidence for a role for sensory neurons and their contained neuropeptides is emerging. Moreover, an apparent increase in neuropeptide immunoreactive nerves occurs in the cervix during pregnancy, maternal serum estrogen levels rise at term and uterine cervix-related L6-S1 dorsal root ganglia (DRG) sensory neurons express estrogen receptor (ER) and neuropeptides. Thus, we sought to test the hypothesis that the neuropeptide substance P (SP) changes biosynthesis and release over pregnancy, that estrogen, acting via the ER pathway, increases synthesis of SP in DRG, and that SP is utilized in cervical ripening at late pregnancy. Using immunohistochemistry, in situ hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and radioimmunoassay (RIA), we investigated coexpression of ER-[alpha]/[beta] and SP; differential expression of ER-[alpha] and -[beta] mRNA in DRG neurons; SP synthesis in DRG; and changes in SP concentration in the cervix, DRG and spinal cord over pregnancy. In addition, the effect of exogenous estrogen on SP synthesis in L6-S1 DRG of ovariectomized rats was examined. SP-immunoreactive neurons expressed ER-[alpha] and ER-[beta]. SP synthesis (expressed as [beta]-PPT mRNA label) was prominent in small DRG neurons. SP concentration increased in the L6-S1 DRG and spinal cord segments,
with a peak at Day 20 of gestation, but decreased in the cervix during the first two trimesters, with a rise over the last trimester to Day 10 levels. SP and ER-[alpha] mRNA synthesis increased in DRG over pregnancy but ER-[beta] mRNA levels were largely unchanged. When ovariectomized rats were treated with exogenous estrogen, SP mRNA synthesis in the DRG increased in a dose-related manner, an effect blocked by ER blocker ICI 182 780. From these results, we postulate that synthesis of SP in L6-S1 DRG and utilization in the cervix increase over pregnancy and this synthesis is under influence of the estrogen-ER system, most likely ER-[alpha]. We postulate that SP may play a role in cervical ripening and, consequently in the birth process.


Monoclonal antibody G15-6A was generated by immunizing mice with Ascaris head extracts. It recognizes an antigen present in a single neuron, with a cell body in the dorsal rectal ganglion, that projects along the ventral cord to the nerve ring. Ascaris extracts were fractionated by HPLC and ammonium sulfate precipitation, and fractions assayed by dot-blotting with antibody G15-6A. A single immunoreactive polypeptide was purified; mass spectrometry showed a molecular weight of 11,542 Da. Partial N-terminal sequencing, followed by cloning of the transcript encoding the peptide, revealed a predicted peptide product comprising 109 amino acids, and a molecular mass of 11,863 Da. The N-terminus of the predicted peptide includes four more amino acids than are found in the isolated product.


http://www.sciencedirect.com/science/article/B6T0M-4D9DFRJ-1/2/ce69d119678dce9375b76ecbe8b95aa

We investigated the involvement of MCH in food intake in barfin flounder. The structure of barfin flounder MCH was determined by cDNA cloning and mass spectrometry. In fasted fish, the MCH gene expression and the number of MCH neurons in the brain were greater than controls. In white-reared fish, the MCH gene expression and the number of MCH neurons in the brain were greater than black-reared fish. Furthermore, white-reared fish grew faster than black-reared fish. These results indicate that a white background stimulated production of MCH and MCH, in turn, enhanced body growth, probably by stimulating food intake.


http://www.sciencedirect.com/science/article/B6T0M-3V8KBK5-4/2/bf0fd690b378e25f0714c73f9d137c55

Pituitary adenylate cyclase activating polypeptide (PACAP), which was isolated from ovine hypothalamic extract, has been shown to have a physiological role in the regulation of insulin or islet functions. In streptozotocin (STZ)-induced diabetic rats, we examined the content of PACAP
immunoreactivity and gene expression of three specific receptors. Four weeks after administration of STZ (50 mg/kg), plasma glucose levels increased 3.3-fold, and plasma insulin levels decreased to one-tenth as compared with the control. The content of PACAP immunoreactivity in the pancreas potently increased by 30%, but the content of vasoactive intestinal polypeptide (VIP) immunoreactivity was not changed. In the other tissues, the content of PACAP immunoreactivity did not significantly change except in the hypothalamus, which showed a 10% increment. In the expression level of PACAP/VIP receptors, semi-quantitative RT-PCR analysis revealed that VIP1/PACAP receptor mRNA significantly increased as compared with the other two types of receptors in the pancreas of STZ-induced diabetic rats. These findings suggest that PACAP and VIP1/PACAP receptor might be involved in the pathophysiology of diabetes mellitus.


mRNA encoding the human NPY Y1 and NPY Y2 receptors were detected in cerebral, meningeal, and coronary arteries using reverse transcriptase-polymerase chain reaction (RT-PCR). In addition, the trigeminal and superior cervical ganglia were positive for both receptors. In some arteries and in SK-N-MC cells only mRNA encoding the NPY Y1 was detected. Besides the expected NPY Y1 PCR products, an additional 97 bp longer amplicon originating from an alternative splicing event was found in most tissues studied. Antibodies directed against the NPY Y1 receptor revealed immunostaining mainly in the smooth muscle layer of blood vessels whereas antibodies against the NPY Y2 receptor showed immunostaining in nerve cell bodies.


http://www.sciencedirect.com/science/article/B6T0M-44V0PX6-D/2/f075162c335694ebf63a1e6b77bcb5a0

The cDNA sequence encoding the CP2 neuropeptide precursor is identified and encodes a single copy of the neuropeptide that is flanked by appropriate processing sites. The distribution of the CP2 precursor mRNA is described and matches the CP2-like immunoreactivity described previously. Single cell RT-PCR independently confirms the presence of CP2 precursor mRNA in selected neurons. MALDI-TOF MS is used to identify additional peptides derived from the CP2 precursor in neuronal somata and nerves, suggesting that the CP2 precursor may give rise to additional bioactive neuropeptides.


http://www.sciencedirect.com/science/article/B6T0M-44V0PX6-G/2/4eff1dc146f2ce0d05bbb6c3140f6f7a

The coding region of 153 amino-acid sorbin, isolated from porcine intestine has been cloned and sequenced in pig, human and rat. The coding region includes 459 bases comprising the 5' region
of 24 bases, the middle region named "sorbin-like sequence" (25-432) and the 3' region (433-459). The peptidic C-terminal segment presents the biological activity: absorption of water and electrolytes from the intestine and gall-bladder. The cDNA homology between the three species was 95%. Three forms of mRNA were found, two major forms (6.5 and 8 Kb) and one minor (4.5 Kb).


http://www.sciencedirect.com/science/article/B6T0M-41KP2XJ-J/2/75771a71c2578b320007de0663d2334a

Glucose-dependent insulinotropic peptide (GIP) potentiates glucose-induced insulin secretion. In addition, GIP has vasoconstrictive or vasodilatory properties depending on the vascular bed affected. In order to assess whether this effect could be related to differences in GIP receptor expression, several different endothelial cell types were examined for GIP receptor expression. GIP receptor splice variants were detected and varied depending on the endothelial cell type. Furthermore, stimulation of these cells with GIP led to cell type dependent differences in activation of the calcium and cAMP signaling pathways. To our knowledge this is the first physiological characterization of receptors for GIP in endothelial cells.


http://www.sciencedirect.com/science/article/B6T0M-47MKCMH-2/2/4d0662deac9dac8418ffe2960286e9ac

Previous studies have confirmed an important role of the undecapeptide substance P (SP) in opioid reward and dependence. It is further shown that the SP N-terminal metabolite SP1-7 may attenuate the intensity of opioid withdrawal in mice. In this study we have investigated the effect of the heptapeptide fragment on the expression of the brain dopamine D2 receptor mRNA and on the withdrawal reaction, as well, in morphine-dependent rats. Male Sprague-Dawley rats were randomly distributed into two groups. Guide cannula was implanted and aimed at the lateral ventricle and animals were subsequently made opioid dependent by two daily injections of morphine (10 mg/kg) for 7 days. Half an hour before naloxone challenge (2 mg/kg) one group of rats received an injection of SP1-7 (28 nmol per rat) and the other, serving as control, was injected with saline through the cannula. Animals were decapitated 4 h following SP1-7 or saline injections. The results indicated that the level of the dopamine D2 receptor transcript was significantly reduced by SP1-7 in nucleus accumbens and frontal cortex but not altered in the striatum. In behavioral tests it was found that the heptapeptide attenuated several somatic withdrawal symptoms. The observed reduction in the receptor transcript in nucleus accumbens and frontal cortex is suggested to reflect an increased dopamine activity in these areas, which in turn may counteract the withdrawal reaction.
An A to G point mutation that results in a serine to glycine amino acid change (S291G) in the acetylcholinesterase (AChE, EC 3.1.1.7) gene was identified previously as associated with azinphosmethyl resistance in Colorado potato beetle due to target site insensitivity. To efficiently validate the detection process of the S291G mutation and base the DNA diagnostic method on direct determination of nucleic acid sequence, a single-stranded conformational polymorphism (SSCP) protocol and a minisequencing reaction were developed. SSCP protocols using a 163-bp DNA template that spans the mutation resulted in an easy, rapid, cheap, and rugged DNA-based diagnostic method, which was capable of separating azinphosmethyl-susceptible and -resistant beetles. For minisequencing, PCR-amplified and biotinylated DNA templates from both susceptible and resistant beetles, which contain the mutation site, were bound to streptavidin-coated microplate strips. Minisequencing was accomplished with a detection primer that annealed adjacent to the point mutation, digoxigenin-labeled dATP, or alternatively, digoxigenin-labeled dUTP and AmpliTaq polymerase. The sequencing reaction added a digoxigenin-labeled dATP only when matched to the biotinylated DNA template (dATP and 3’GGTCA5’). Digoxigenin-labeled DNA was detected using peroxidase-conjugated digoxigenin antibodies and quantitated as optical density (OD) at 450 nm in a microplate reader. The OD readings obtained with digoxigenin-labeled dATP in the presence of susceptible AChE DNA template was 0.319 +/- 0.05, which was significantly higher than that obtained in the presence of the azinphosmethyl-resistant template (0.031 +/- 0.018) (P < 0.001). These highly significant results agree well with the susceptibility of AChE from individual insects as judged by AChE inhibition by azinphosmethyl-oxon and further support the contention that A to G point mutation, which occurs only in AChE gene of azinphosmethyl-resistant beetles, is responsible for enzyme insensitivity. Compared with SSCP, the minisequencing reaction provides a direct means to validate this specific point mutation. Coupling minisequencing with the ease and durability of SSCP will allow us to determine the presence or absence of the S291G mutation in an efficient and unambiguous manner. As such, similar approaches could be used to validate point mutations in any resistant strain of insect.

Pharmacological Research (2)


The keggin-type heteropolyoxotungstate K7[PTi2W10O40].6H2O (PM-19) is a potent polyoxometalate (PM) inhibitor of the replication of herpes simplex virus (HSV). Pretreatment of Vero cells with PM-19 prior to HSV-2 infection enhanced the antiviral potency of PM-19 almost 10-fold compared with treatment of the cells only after infection. The pretreatment effect of PM-19 is called 'the memory effect'. The memory effect was reflected by inhibition of plaque formation.
and decrease of intracellular virus DNA quantity, and was strongest when PM-19 was present during the penetration stage of HSV-2 infection. The effect was maintained under conditions of fusion induced by polyethyleneglycol treatment. This suggests that PM-19 does not act at the fusion stage of infection. Using the infectious center assay method, it was clarified that a second round of infection was inhibited by about 30% in the presence of PM-19 at the penetration stage compared with the virus control in nontreated cells. The inhibition was enhanced to about 60% by PM-19 pretreatment prior to infection. This suggests that PM-19 pretreatment of the cells protects them against HSV-2 infection.


http://www.sciencedirect.com/science/article/B6WP9-473VN84-C/2/3f0af8919ff5896e2eb79facf97d270f

The effect of pentoxifylline (PTX), a methylxanthine derivative, on collagen induction and secretion and on the production of mRNA of two fibrogenic cytokines: interleukin-6 and transforming growth factor-β1 (IL-6 and TGF-[β]) in a rat hepatic stellate cell line (CFSC-2G) exposed to acetaldehyde was studied. CFSC-2G cells were treated with 175 [μ]M acetaldehyde for 24 h. The cells were then exposed to a medium containing 200 [μ]M PTX. Collagen secretion, increased 2.6 times in acetaldehyde treated cells. Cells exposed to acetaldehyde and treated with PTX diminished collagen secretion to control values and decreased [α1(I)] collagen mRNA by 15%. Reverse transcriptase-polymerase chain reaction (RT-PCR) assays of TGF-[β]1 mRNA showed no variation in different experimental conditions. However, PTX induced a decrease of 32% in IL-6 mRNA in acetaldehyde-treated cells. CFSC-2G cells treated with anti-IL-6 monoclonal antibody, 15 min before acetaldehyde was added, did not present an increase in [α1(I)] collagen mRNA. These results show that PTX inhibits the expression of [α1(I)] collagen via the inhibition of IL-6 in acetaldehyde treated cells. The effect herein reported on IL-6 and [α1(I)] collagen mRNA adds to the previously described effect of PTX, which could be useful in the fibrogenic process induced by acetaldehyde.

Physiol Genomics (13)


http://physiolgenomics.physiology.org/cgi/content/abstract/00025.2005v1

Quantitative gene expression data are often normalised to the expression levels of control or so-called 'housekeeping' genes. An inherent assumption in the use of housekeeping genes is that expression of the genes remains constant in the cells or tissues under investigation. Although exceptions to this assumption are well documented, housekeeping genes are of value in fully characterised systems. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data. To investigate the value of GAPDH as a housekeeping gene in human tissues, the expression of
GAPDH mRNA was measured in a panel of 72 different pathologically normal human tissue types. Measurements were obtained from 371,088 multiplexed, quantitative real-time reverse transcription polymerase chain reactions (Q-RTPCRs) with specific target genes. Significant differences in the expression levels of GAPDH mRNA were observed between tissue types, and between donors of the same tissue. A 15-fold difference in GAPDH mRNA copy numbers was observed between the highest and lowest expressing tissue types, skeletal muscle and breast, respectively. No specific effect of either age or gender was observed on GAPDH mRNA expression. These data provide an extensive analysis of GAPDH mRNA expression in human tissues, and confirm previous reports of the marked variability of GAPDH expression between tissue types. These data establish comparative levels of expression, and can be used to add value to gene expression data in which GAPDH is used as the internal control.


A fast inwardly rectifying current has been observed in some of the sensory cells (hair cells) of the inner ear of several species. While the current was presumed to be an IKir current, contradictory evidence existed as to whether the cloned channel actually belonged to the Kir2.0 subfamily of potassium inward rectifiers. In this paper, we report for the first time converging evidence from electrophysiological, biochemical, immunohistochemical, and genetic studies that show that the Kir2.1 channel carries the fast inwardly rectifying currents found in pigeon vestibular hair cells. Following cytoplasm extraction from single type II and multiple pigeon vestibular hair cells, mRNA was reverse transcribed, amplified, and sequenced. The open reading frame (ORF), consisting of a 1,284-bp nucleotide sequence, showed 94, 85, and 83% identity with Kir2.1 subunit sequences from chick lens, Kir2 sequences from human heart, and a mouse macrophage cell line, respectively. Phylogenetic analyses revealed that pKir2.1 formed an immediate node with hKir2.1 but not with hKir2.2-2.4. Hair cells (type I and type II) and supporting cells in the sensory epithelium reacted positively with a Kir2.1 antibody. The whole cell current recorded in oocytes and CHO cells, transfected with pigeon hair cell Kir2.1 (pKir2.1), demonstrated blockage by Ba2+ and sensitivity to changing K+ concentration. The mean single-channel linear slope conductance in transfected CHO cells was 29 pS. The open dwell time was long (~300 ms at -100 mV), and the closed dwell time was short (~34 ms at -100 mV). Multistates ranging from 3-6 were noted in some single-channel responses. All of the above features have been described for other Kir2.1 channels. Current clamp studies of native pigeon vestibular hair cells illustrated possible physiological roles of the channel and showed that blockage of the channel by Ba2+ depolarized the resting membrane potential by ~30 mV. Negative currents hyperpolarized the membrane ~20 mV before block but ~60 mV following block. RT-PCR studies revealed that the pKir2.1 channels found in pigeon vestibular hair cells were also present in pigeon vestibular nerve, vestibular ganglion, lens, neck muscle, brain (brain stem, cerebellum and optic tectum), liver, and heart.


We previously demonstrated expression of full-length transcripts for sublingual mucin apoprotein, Muc19, of ~24 kb (Fallon MA, Latchney LR, Hand AR, Johar A, Denny PA, Georgel PT, Denny PC, and Culp DJ. Physiol Genomics 14: 95-106, 2003). We now describe the complete sequence
and genomic organization of the apomucin encoded by 43 exons. Southern analyses indicate a central exon of ~18 kb containing 36 tandem repeats, each encoding 163 residues rich in serine and threonine. Full-length transcripts are an estimated 22,795 bp in length that span 106 kb of genomic DNA. The transcriptional start site is 24 bp downstream of a TATA box and 42 bp upstream of the conceptual translational start codon. The putative apoprotein has an estimated mass of 693.4 kDa and contains 7,524 amino acids (80% serine, threonine, glycine, alanine, and proline). We present a model for rat Muc19 transcripts and compare the conceptually translated Muc19 proteins for mouse, rat, pig, and the 3’ end of human Muc19. Conserved among these apoproteins are a signal peptide, a large tandem repeat region, von Willebrand factor type C and D domains, a trypsin inhibitor-like Cys-rich domain, and a COOH-terminal cystine knot-like domain. Southern blot analyses indicate transcripts for Muc19 and Smgc (submandibular gland protein C) are splice variants of a larger gene, Muc19/Smgc. Comparative Northern analyses between the major salivary glands demonstrate highly selective Muc19 expression in neonatal and adult sublingual glands, whereas Smgc is expressed in neonatal submandibular and sublingual glands. Regulation of Muc19/Smgc gene expression is discussed with respect to alternative splicing and mucous cell cytodifferentiation.

http://physiolgenomics.physiology.org/cgi/content/abstract/15/2/127

Estrogen induction of uterine wet weight provides an excellent model to investigate relationships between changes in global gene expression and well-characterized physiological responses. In this study, time course microarray GeneChip data were analyzed using a novel approach to identify temporal changes in uterine gene expression following treatment of immature ovariectomized C57BL/6 mice with 0.1 mg/kg 17α-ethynylestradiol. Functional gene annotation information from public databases facilitated the association of changes in gene expression with physiological outcomes, which allowed detailed mechanistic inferences to be drawn regarding cell cycle control and proliferation, transcription and translation, structural tissue remodeling, and immunologic responses. These systematic approaches confirm previously established responses, identify novel estrogen-regulated transcriptional effects, and disclose the coordinated activation of multiple modes of action that support the uterotrophic response elicited by estrogen. In particular, it was possible to elucidate the physiological significance of the dramatic induction of arginase, a classic estrogenic response, by elucidating its mechanistic relevance and delineating the role of arginine and ornithine utilization in the estrogen-stimulated induction of uterine wet weight.

http://physiolgenomics.physiology.org/cgi/content/abstract/00278.2004v1

Angiogenesis is a complex multicellular process requiring the orchestration of many events including migration, alignment, proliferation, lumen formation, remodeling and maturation. Such complexity indicates that not only individual genes but also entire signalling pathways will be crucial in angiogenesis. In order to define an angiogenic blueprint of regulated genes, we utilized our well characterized 3D collagen gel model of in vitro angiogenesis, in which the majority of cells synchronously progress through defined morphological stages culminating in the formation of capillary tubes. We developed a comprehensive three tiered approach using microarray
analysis which has allowed identification of genes known to be involved in angiogenesis, genes hitherto unlinked to angiogenesis as well as novel genes, and has proven especially useful for genes where the magnitude of change is small. Of interest is the ability to recognize complete signalling pathways which are regulated and genes clustering into ontological groups implicating the functional importance of particular processes. We have shown that consecutive members of the mitogen activated protein kinase (MAPK) and leukemia inhibitory factor (LIF) signalling pathways are altered at the mRNA level during in vitro angiogenesis. Thus, at least for the MAPK pathway, mRNA changes as well as the phosphorylation changes of these gene products may be important in the control of blood vessel morphogenesis. Furthermore, in this study, we demonstrate the power of virtual northern blot analysis, as an alternative to quantitative reverse transcription polymerase chain reaction (Q-RT-PCR), for measuring the magnitudes of differential gene expression.

http://physiolgenomics.physiology.org/cgi/content/abstract/21/2/152

Erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinases and their ligands, ephrins, are involved in embryogenesis and oncogenesis by mediating cell adhesion and migration. Although ephrins can be induced by bacterial LPS in vitro, whether they are involved in inflammation in vivo is unknown. Using differential mRNA display, we found that a febrigenic dose of LPS (50 \(\mu\)g/kg iv) induces a strong transcriptional upregulation of ephrin-A1 in rat liver. We confirmed this finding by real-time RT-PCR. We then quantified the mRNA expression of different ephrins and Eph receptors at phases 1-3 of LPS fever in different organs. Febrile phases 2 (90 min post-LPS) and 3 (300 min) were characterized by robust upregulation (up to 16-fold) and downregulation (up to 21-fold) of several ephrins and Eph receptors. With the exception of EphA2, which showed upregulation in the brain at phase 2, expressional changes of Eph receptors and ephrins were limited to the LPS-processing organs: liver and lung. Characteristic, counter-directed changes in expressional regulation of Eph receptors and their corresponding ligands were found: upregulation of EphA2, downregulation of ephrin-A1 in the liver and lung at phase 2; downregulation of EphB3, upregulation of ephrin-B2 in the liver at phase 2; downregulation of EphA1 and EphA3, upregulation of ephrins-A1 and -A3 in liver at phase 3. In the liver, transcriptional changes of EphA2 and EphB3 at phase 2 were confirmed at protein level. These coordinated, phase-specific responses suggest that different sets of ephrins and Eph receptors may be involved in cellular events (such as disruption of tissue barriers and leukocyte transmigration) underlying different stages of systemic inflammatory response to LPS.

http://physiolgenomics.physiology.org/cgi/content/abstract/00018.2005v1

Disturbed gene expression may disrupt the normal process of repair and lead to pathologic situations resulting in excessive scarring. To prevent and treat impaired healing it is necessary to first define baseline gene expression during normal repair. The objective of this study was to compare gene expression in normal intact skin (IS) and wound biopsies (WM), using suppression subtractive hybridization (SSH) to identify genes differentially expressed during wound repair in horses. Tissue samples included both normal intact skin and biopsies from 7 day old wounds. IS cDNAs were subtracted from WM cDNAs to establish a subtracted (WM-IS) CDNA library. 226 non-redundant cDNAs were identified. Detection of genes previously shown to be expressed 7
days following trauma, including COL1A2, ANXA2, COL6A3, ACTB, FGF-7, LAMR1, MMP1, SPARC and TIMP-2, supported the validity of the experimental design. An RTPCR assay confirmed an increase or induction of the cDNAs of specific genes (COL1A2, MMP1, DSPG2, CD68, CD163 and ADAM9) within wound biopsies. Among these, COL1A2 and MMP1 had previously been documented in horses. 68.8% of the cDNAs had not previously been attributed a role during wound repair, of which SSAT, SERPINB10 and SNX9 were highly expressed and whose known functions in other processes made them potential candidates in regulating the proliferative response to wounding. In conclusion, we have identified novel genes that are differentially expressed in equine wound biopsies and which may modulate repair. Future experiments must correlate changes in mRNA levels for precise molecules with spatio-temporal protein expression within tissues.


http://physiolgenomics.physiology.org/cgi/content/abstract/16/2/275

(beta)-Adrenergic receptor agonists (BA) stimulate skeletal muscle growth. However, downstream signaling pathways that facilitate this effect remain poorly defined. Objectives of this study were to identify genes differentially expressed after administration of a novel BA and to evaluate the expression of one of those genes in additional models of skeletal muscle growth. Differentially expressed gene fragments were identified through differential display of skeletal muscle biopsies from five steers 24 h after administration of the BA. Five gene fragments designated DD53, DD143, DD163, DD209, and DD214 were identified. Tissue distribution of these genes was evaluated by RT-PCR. While DD53, DD163, DD209, and DD214 were expressed across tissues, DD143 mRNA expression was most abundant in skeletal muscle. DD143, later identified as bovine ASB15, was evaluated in rats following administration of anabolic compounds. Thirteen 7-wk-old female rats were randomly assigned to each of four treatment groups including: control, clenbuterol, trenbolone acetate (TBA), and growth hormone (GH). Changes in rat Asb-15 mRNA were measured at 30 min, 12 h, and 24 h following intraperitoneal injections of each compound. Clenbuterol treatment decreased Asb-15 mRNA in skeletal muscle at 12 and 24 h (P < 0.01) and also decreased mRNA in lung at 12 h (P < 0.05). TBA and GH treatments did not alter Asb-15 mRNA in any of the tissues evaluated (P > 0.10). These results are the first to associate an Asb gene family member with muscle growth or BA administration and suggest a potential role for ASB15 in (beta)-agonist-induced skeletal muscle hypertrophy.


http://physiolgenomics.physiology.org/cgi/content/abstract/21/2/212

Wilms' tumor gene (WT1) is important for nephrogenesis and gonadal growth. WT1 mutations cause Denys-Drash and Frasier syndromes, which are characterized by glomerular scarring. To test whether genetic variations in WT1 and WIT1 (gene immediately 5' to WT1) associate with focal segmental glomerulosclerosis (FSGS), patients with biopsy-proven idiopathic and HIV-1-associated FSGS were enrolled in a multicenter study. We genotyped SNP rs6508 located in WT1 exon 1, three SNPs (rs2301250, rs2301252, rs2301254) in the promoter shared by WT1 and WIT1, rs2234590 in exon 6, rs2234591 in intron 6, rs16754 in exon 7, and rs1799937 in intron 9 of WT1. Cases (n = 218) and controls (n = 281) were compared in the African American population. Stratification by HIV-1 infection status showed that SNPs rs6508, rs2301254, and
rs1799937 were significantly associated with FSGS [rs6508 odds ratio (OR) 1.82, P = 0.006; rs2301254 OR 1.65, P = 0.049; rs1799937 OR 1.91, P = 0.005] in the non-HIV-1 group and rs2234591 (OR 0.234, P = 0.011) in the HIV-1 group. Haplotype analyses in the population revealed that seven SNPs were associated with FSGS; five SNPs had the highest contingency score [-\log_{10}(P value) = 13.57] in the HIV-1 group. This association could not be explained by population substructure. We conclude that SNPs in WT1 and WIT1 genes are significantly associated with FSGS, suggesting that variants in these genes may mediate pathogenesis by altering WT1 function. Furthermore, HIV-1 infection status interacts with genetic variations in both genes to influence this phenotype. We speculate that nephropathy liability alleles in WT1 pathway genes cause podocyte dysfunction and glomerular scarring.


http://physiolgenomics.physiology.org/cgi/content/abstract/20/1/21

Piscirickettsia salmonis is the intracellular bacterium that causes salmonid rickettsial septicemia, an infectious disease that kills millions of farmed fish each year. The mechanisms used by P. salmonis to survive and replicate within host cells are not known. Piscirickettsiosis causes severe necrosis of hematopoietic kidney. Microarray-based experiments with QPCR validation were used to identify Atlantic salmon macrophage and hematopoietic kidney genes differentially transcribed in response to P. salmonis infection. Infections were confirmed by microscopy and RT-PCR with pathogen-specific primers. In infected salmon macrophages, 71 different transcripts were upregulated and 31 different transcripts were downregulated. In infected hematopoietic kidney, 30 different transcripts were upregulated and 39 different transcripts were downregulated. Ten antioxidant genes, including glutathione S-transferase, glutathione reductase, glutathione peroxidase, and cytochrome b558 (alpha- and (beta)-subunits, were upregulated in infected macrophages but not in infected hematopoietic kidney. Changes in redox status of infected macrophages may allow these cells to tolerate P. salmonis infection, raising the possibility that treatment with antioxidants may reduce hematopoietic tissue damage caused by this rickettsial infection. The downregulation of transcripts involved in adaptive immune responses (e.g., T cell receptor (alpha)-chain and C-C chemokine receptor 7) in infected hematopoietic kidney but not in infected macrophages may contribute to infection-induced kidney tissue damage. Molecular biomarkers of P. salmonis infection, characterized by immune-relevant functional annotations and high fold differences in expression between infected and noninfected samples, may aid in the development of anti-piscirickettsial vaccines and therapeutics.


http://physiolgenomics.physiology.org/cgi/content/abstract/12/3/175

The purpose of this study was to investigate the effects of ovarian hormones on gene expression in the vascular wall. Our approach employed an RT-PCR-based cloning strategy of DNA differential display analysis and verification/confirmation of differential expression by semi-quantitative PCR and real-time PCR. mRNA analysis of normal aortas from intact and ovariectomized female C57BL/6J mice, showed altered expression of 20 genes with significant (>70%) sequence homology to known genes. Eight were selected for further study based on the genes' known function and potential relevance to vascular physiology. Differential expression of mRNA for three genes was confirmed by both semi-quantitative and real-time RT-PCR using gene-specific primers. Ovariectomy downregulated expression of elongation factor-1(alpha) (3.5-
fold), ganglioside-induced differentiation associated protein (8.2-fold), and NADH:ubiquinone oxidoreductase (3.8-fold). Thus, in normal mouse aortas, ovariectomy resulted in significant differential downregulation of a number of vascular genes important to vascular cell growth and angiogenesis, cellular differentiation, and mitochondrial energy metabolism, respectively. These studies have implications for our understanding of hormonal regulation of vascular gene expression and the therapeutic targeting of specific vascular genetic sequences by female sex steroid hormones.


http://physiolgenomics.physiology.org/cgi/content/abstract/14/1/83

Hypertension is a complex trait with multiple genetic determinants. A previous genome-wide linkage study of systolic blood pressure in a mouse genetic backcross implicated a region of chromosome 13 (LOD = 3.3 at 16.0 cM) as a determinant of blood pressure differences between a hereditary low blood pressure strain of Mus musculus (BPL/1) and Mus spretus (SPRET); at this locus, the unexpected effect of the BPL/1 allele was to increase blood pressure. A plausible candidate locus encoding angiotensin II receptor isoform 1a (Agtr1a) is also located at 16.0 cM on chromosome 13. We therefore investigated structural and functional differences at Agtr1a between BPL/1 and SPRET, as well as the BPH/2 strain. Resequesting Agtr1a in the three strains established the exon/intron and proximal promoter structure of the mouse gene. Coding exon 3 spanned 1,960 bp (with 26 SNPs), including the 1,077-bp/359-amino acid ORF (with 5 cSNPs, all of which were synonymous). Promoter sequences revealed a consensus TATA box, conserved G/C-rich regions, and a striking, lengthy simple sequence repeat region, composed of di-, tri-, tetra-, and penta-nucleotide repeats, whose overall length varied markedly among the strains. Twenty-five other SNPs and three single nucleotide deletions differentiated the strains' promoters, six of which were in likely functional promoter motifs. Agtr1a mRNA abundance in the adrenal gland in vivo was greater (P < 0.05) in BPL/1 than SPRET, consistent with the predicted effect of the BPL/1 allele to confer higher blood pressure. When Agtr1a promoters were subcloned into luciferase reporter plasmids and transfected into PC12 chromaffin cells, basal promoter expression was higher (P < 0.001) in BPL/1 than in SPRET, consistent with the endogenous mRNA results. In summary, Agtr1a on chromosome 13 is highly polymorphic between mouse strains, although the amino acid sequence specified by the ORF is invariant, even across mouse species. We conclude that polymorphisms in the Agtr1a promoter account for differences in gene expression in vivo between BPL/1 and SPRET, in a way consistent with the effects of alleles at this locus on chromosome 13 to affect blood pressure in the mouse genome-wide linkage study.


http://physiolgenomics.physiology.org/cgi/content/abstract/17/2/122

The full extent to which 1,25-dihydroxyvitamin D3 affects gene expression in human intestinal epithelial cells is unknown. We used oligonucleotide arrays to catalog vitamin D-induced changes in gene expression in Caco-2 cells, a human colon carcinoma cell line. Five paired sets of Caco-2 cell cultures were subjected to either control conditions or 1,25-dihydroxyvitamin D (10-7 mol/l x 24 h), and RNA was analyzed on an Affymetrix cDNA array containing 12,625 human sequences. Only 13 sequences representing 12 distinct genes exhibited statistically significant changes in expression of twofold or greater and were also called as "present" or "marginal" by the array-
reading software in all five experiments. Genes regulated by 1,25-dihydroxyvitamin D included two previously known genes (25-hydroxyvitamin D-24-hydroxylase and amphiregulin) and 10 genes (sorcin, Gem, adaptin-(gamma), TIG1, CEACAM6, carbonic anhydrase XII, junB, ceruloplasmin, and two unidentified sequences) that were novel. We tested and independently confirmed the effect of 1,25-dihydroxyvitamin D on 11 of these genes by RT-PCR. Increased protein expression was tested and confirmed in two of the novel 1,25-dihydroxyvitamin D-regulated genes, ceruloplasmin and sorcin. The known function of these genes suggests that many of them could be involved in the antiproliferative effects of 1,25-dihydroxyvitamin D3.

Physiological and Molecular Plant Pathology (3)


http://www.sciencedirect.com/science/article/B6WPC-4CBV2VF-1/2/bd4e8d3802e7e299d9adb10d621b4d06

Grain mold of Sorghum bicolor is one of the leading constraints for the production of optimum quality sorghum grain worldwide. Differences in mold levels among different varieties grown in the same environment imply that genes play a role in controlling mold severity. In order to determine if genes that function in active defense responses also affect molding, panicles of resistant and susceptible cultivars were inoculated at anthesis with conidial suspensions of Fusarium thapsinum and Curvularia lunata, the fungi most often found in naturally infected grain. RNA was extracted from the immature floral tissues at various times following inoculation. Levels of mRNA for four known defense-response genes, phenylalanine ammonia lyase (PAL1-1), chalcone synthase (CHS2G), [beta]-1,3-glucanase (GLUC2-1) and chitinase (CHIT25-1) were examined by hybridization to PCR generated clones of the respective genes. Expression of each gene increased rapidly following inoculation with either fungus. Although differences were seen in response to the two pathogens, the general pattern was similar in resistant and susceptible cultivars. The results imply that factors other than the level or timing of active defense responses account for the cultivar differences seen when the plants are challenged at the time of flowering.


http://www.sciencedirect.com/science/article/B6WPC-49CSYXY-2/2/94073937026ab563e6b2ce479cb210b5

The WS-Y isolate of Watermelon silver mottle virus (WSMoV) causes severe necrosis in Tetragonia expansa. To determine the RNA segment that induces symptoms, genome reassortants between WS-Y and an isolate causing mild mottle, WS-O, were generated. The origin of each segment in the reassortants was identified by RT-PCR and subsequent restriction enzyme analysis of the amplified fragments. Thirty genome reassortants were isolated from co-infected T. expansa plants. The reassortants with the S RNA segment of WS-Y caused severe necrosis, while those with the S RNA segment of WS-O caused a mild mottle; hence, the S RNA
determined symptom expression. The incidence of reassortants was disproportional among genotypes. The most frequent genome reassortant possesses the L RNA of WS-Y, the M RNA of WS-O and the S RNA of WS-Y. A similar ratio of genotypes was found in isolates of local lesions on Chenopodium quinoa. These results strongly suggested that competition occurred independently between the individual RNA segments in a co-infected T. expansa plant, not between isolates.


http://www.sciencedirect.com/science/article/B6WPC-4F2VS6B-1/2/8e3b8bdc0ed657eb44a45a94194edcd1

A gene encoding the ATP-binding cassette (ABC) transporter FcABC1 was characterised in Fusarium culmorum. It is homologous to two transporters, Magnaporthe grisea ABC1 and Gibberella pulicaris ABC1, that play a role in plant pathogenesis. Transcripts of FcABC1 were present during infection of barley roots. A FcABC1 replacement mutant was produced and in a point inoculation experiment of wheat heads, the mutant displayed reduced aggressiveness, indicating that FcABC1 has a function during infection of plant tissue. It is hypothesised that wheat contains an antifungal compound that inhibits the spread of the fungus in the plant and to which FcABC1 confers resistance.

Physiology & Behavior (2)


http://www.sciencedirect.com/science/article/B6T0P-45KWD47-2/2/9156c6770f223f557848ed90853db2be

Animals tend to maintain a lower body weight for an extended period after leptin administration has ended. This may be due to an enhancement of metabolic rate that persists after treatment withdrawal. Our objectives were to determine the period of leptin influence, when injected intracerebroventricularly (icv), on food intake, body weight, and energy expenditure. Additionally, the relationship between expressions of UCP1, UCP2, and UCP3 in different adipose tissues and heat production (HP) was assessed. Twenty-four adult male Sprague-Dawley rats were injected intracerebroventricularly with either 10 [mu]g mouse leptin or 10 [mu]l vehicle once per day for 4 days. At 24 h after the last injection, one group was killed while the other was placed in calorimetry chambers and monitored for 21 days of recovery. Leptin-injected rats exhibited an overshoot of food intake and respiratory quotient (RQ) during recovery, but body weight remained significantly lower up to 6 days. HP decreased in both groups over time but remained higher in the leptin group through recovery. However, retained energy (RE) was significantly greater than control for about 8 days. Overall, UCP expression was reduced at the end of recovery in parallel with the decline in HP. Brown adipose tissue (BAT) was the most responsive to leptin administration by dramatically changing UCP1 and UCP3 mRNA levels. Our data show that leptin has extended effects on energy expenditure but relieves control on food intake and RQ after
treatment withdrawal. This translated into a reduced positive energy balance that slowed body weight recovery.


http://www.sciencedirect.com/science/article/B6T0P-40TR8DW-G/2/b180ccb9f09d41f0371c2036f9b39a89

Sexual dimorphism of the rodent brain is manifested by the epigenetic action of gonadal steroids. Our previous research identified the granulin (grn) precursor gene as a sex steroid-inducible gene, which was shown to be expressed more abundantly in male than female neonates at the mediobasal hypothalamic area. Grn is a 6-kDa polypeptide promoting or inhibiting the growth of epithelial cells and hematocytes in vitro. In this study, effects on male sexual behavior of male were pursued under conditions in which grn gene expression was suppressed during the critical period. To this end, an antisense oligodeoxynucleotide (ODN) of the grn precursor gene was designed, incorporated into inactivated Sendai virus (HVJ)-liposome complexes, and infused into the third ventricle of 2-day-old male rats. Two different control treatments were used: the first consisted of a control sequence ODN that had little homology to known mRNAs; the second of vehicle (HVJ-liposome) alone. After maturation, animals treated with antisense ODN of grn displayed significantly lower scores than control males on various parameters assessing sexual behavior; i.e., mount, intromission, and ejaculation. The antisense ODN, however, did not affect body growth or serum concentrations of testosterone and luteinizing hormone. Further, there was no significant difference in the volume of the sexual dimorphic nucleus of the preoptic area between antisense ODN-treated and control animals. It was shown that inadequate expression of the grn gene in the brain of male neonatal rats during the critical period suppressed the induction of some type of male sexual behavior, suggesting the grn was involved in the process of masculinization of the rat brain.

Phytochemistry (7)


http://www.sciencedirect.com/science/article/B6TH7-3V9CVG6-1T/2/63949212e245f1aca75e3f9fe5bc37dc

Seeds of the common bean Phaseolus vulgaris and the tepary bean (P. acutifolius) contain a family of plant defence proteins that includes phytohaemagglutinin (PHA), arcelin and [alpha]-amylase inhibitor ([alpha]AI). These homologous proteins differ by the absence of short loops at the surface of the protein and by the presence of a proteolytic processing site (Asn77) that allows [alpha]AI to be post-translationally cleaved and activated. We now report the derived amino acid sequence of two amylase inhibitor-like (AIL) proteins that are not proteolytically processed, although they have the typical processing site. One protein is from the common bean, and the other from the tepary bean. On a dendrogram, these proteins are grouped with [alpha]AIs rather than with the arcelins or lectins. [alpha]AI differs from AIL primarily by the deletion of a 15-amino-
acid segment from the middle of the AIL sequence. When [alpha]AI is expressed in tobacco, it is proteolytically processed to form an active molecule. However, AIL sequences are not processed. We suggest that the AIL proteins may be an intermediate in the evolution of an active [alpha]AI.


http://www.sciencedirect.com/science/article/B6TH7-3S9M8WM-1J/2/fd9b343d53e750a111c1e709936fb5d9d

A new cDNA clone coding for an aspartic proteinase inhibitor homologue was isolated from a potato tuber cDNA library. Southern blot analysis was used to study the structural diversity of the aspartic proteinase inhibitor gene family in several species of the Solanaceae. The existence of sequence-homologous genes was confirmed in the genomic DNA of different potato cultivars (Solanum tuberosum L. cv. Desiree, Pentland Squire and Igor), tomato (Lycopersicon esculentum Mill.), aubergine (S. melongena L.) and a wild type of bittersweet (S. dulcamara L.). Northern blot hybridization of total RNA, isolated from leaves under non-stress conditions, of different solanaceous species and of potato tubers showed that the gene transcripts encoding aspartic proteinase inhibitors occur mainly in potato tubers. The presence of several cathepsin D inhibitor isoforms has been detected at the protein level. At least four isoforms were isolated by affinity chromatography on cathepsin D-Sepharose and characterized. Additionally, exogenous treatment of potato plantlets by jasmonic acid (JA) over a wide range of concentrations (0-100 [mu]M) was performed in a stem node culture in vitro. We demonstrated that the expression of aspartic proteinase inhibitor mRNA was drastically induced in potato shoots at concentrations of 50-100 [mu]M JA.


http://www.sciencedirect.com/science/article/B6TH7-481DXX0X/2/4c20bdcd944811da39826bc00cf49cc6

Quality traits of raspberry fruits such as aroma and color derive in part from the polyketide derivatives, benzalacetone and dihydrochalcone, respectively. The formation of these metabolites during fruit ripening is the result of the activity of polyketide synthases (PKS), benzalacetone synthase and chalcone synthase (CHS), during fruit development. To gain an understanding of the regulation of these multiple PKSs during fruit ripening, we have characterized the repertoire of Rubus PKS genes and studied their expression patterns during fruit ripening. Using a PCR-based homology search, a family of ten PKS genes (Ripks1-10) sharing 82-98% nucleotide sequence identity was identified in the Rubus idaeus genome. Low stringency screening of a ripening fruit-specific cDNA library, identified three groups of PKS cDNAs. Group 1 and 2 cDNAs were also represented in the PCR amplified products, while group 3 represented a new class of Rubus PKS gene. The Rubus PKS gene-family thus consists of at least eleven members. The three cDNAs exhibit distinct tissue-specific and developmentally regulated patterns of expression. Ripks5 has high constitutive levels of expression in all organs, including developing flowers and fruits, while Ripks6 and Ripks11 expression is consistent with developmental and tissue-specific regulation in various organs. The recombinant proteins encoded by the three Ripks cDNAs showed a typical CHS-type PKS activity. While phylogenetic analysis placed the three Rubus PKSs in one cluster, suggesting a recent duplication event, their distinct expression patterns suggest that their regulation, and thus function(s), has evolved independently of the structural genes themselves.
A cDNA encoding one of the phenylalanine ammonia-lyase genes from Populus trichocarpa x deltoides was inserted into a baculovirus expression vector and the PAL protein was successfully expressed in insect cell cultures. High levels of active holoenzyme were obtained that could be purified in a single chromatographic step. Site-directed mutagenesis and expression of the mutant enzyme confirmed that conversion of the putative active site serine202 residue to alanine is sufficient to destroy the catalytic activity of PAL.

Petunia hybrida and Citrus paradisi have significantly different flavonoid accumulation patterns. Petunia sp. tend to accumulate flavonol glycosides and anthocyanins while Citrus paradisi is known for its accumulation of flavanone diglycosides. One possible point of regulation of flavanone metabolism is flavanone 3-hydroxylase (F3H) expression. To test whether this is a key factor in the different flavanone usage by Petunia hybrida and Citrus paradisi, F3H mRNA expression in seedlings of different developmental stages was measured using semi-quantitative RT-PCR. Primers were designed to conserved regions of F3H and used to amplify an approximately 350 bp segment for quantitation by PhosphorImaging. Primary leaves of 32 day old grapefruit seedlings and a grapefruit flower bud had the highest levels of F3H mRNA expression. Petunia seedlings had much lower levels of F3H mRNA expression relative to grapefruit. The highest expression in petunia was in primary leaves and roots of 65 day old seedlings. These results indicate that preferential use of naringenin for production of high levels of flavanone glycosides in young grapefruit leaves cannot be attributed to decreased F3H mRNA expression.

Greater celandine (Chelidonium majus L.) has traditional uses in European and Chinese herbal
medicine. In the plant sap significant inhibitory activity against papain was observed. A cysteine proteinase inhibitor, named chelidocystatin, was isolated from the plant using papain Sepharose affinity chromatography followed by gel filtration and ion-exchange chromatography. Chelidocystatin showed a Mr of 10 000 on SDS-PAGE with the pl of 9.3, and was a strong inhibitor of cathepsin L (Ki=5.6 x 10-11 M), papain (Ki=1.1 x 10-10 M) and cathepsin H (Ki=7.5 x 10-9 M). The complete amino acid sequence of the protein was obtained with N-terminal sequencing and sequencing of the peptides after digestion of the protein. Moreover, a major part of the sequence was verified by molecular cloning. The conserved glycine residue at the N-terminal region and the QVVAG motif, which are both believed to be involved in the inhibitory activity, indicate that it is a member of the cystatin superfamily. The amino acid sequence of chelidocystatin shows a high degree of homology with cysteine proteinase inhibitors belonging to the phytocystatin group, especially with the recently described carrot and sunflower phytocystatins with which it shares 57% and 54% homology, respectively.

Phytomedicine (1)


http://www.sciencedirect.com/science/article/B7GVW-4CRPN61-4/2/1af9f35083976de9add124cb5144338c

Belamcanda chinensis (BC) belongs to the family of iridaceae and the isoflavone tectorigenin has been isolated from the rhizome of this plant. Whether this isoflavone has estrogenic, possibly selective estrogen receptor modulator activities and if so, whether they are mediated via the estrogen receptor [alpha] or [beta] is unknown at present. Therefore, we performed binding studies with recombinant human ER[alpha] and ER[beta] to show that tectorigenin binds to both receptor subtypes. In ER[alpha]-expressing MCF7 and ER[beta]-expressing MDA-MB231 reporter gene transfected cells tectorigenin causes transactivation. When given intravenously to ovariectomized (ovx) rats, it inhibits pulsatile pituitary LH secretion. In postmenopausal women estrogen-unopposed LH pulses correlate with hot flushes. Therefore, suppression of pulsatile LH secretion may be beneficial in women suffering from hot flushes. Upon chronic application to ovx rats a BC extract containing 5% Belamcanda at a daily dose of 33 mg or 130 mg of the extract had no effect on uterine weight or on estrogen-regulated uterine gene expression while estrogenic effects in the bone, on bone mineral density of the metaphysis of the tibia could be established. Hence, tectorigenin may have antosteoporotic effects also in postmenopausal women. Immunohistochemical staining of proliferating cell nuclear antigen—a proliferation marker-in the mammary gland did not indicate a mammotrophic effect of the tectorigenin-containing BC extract at both tested doses. In summary, tectorigenin or the B. chinensis extract containing tectorigenin had a strong hypothalamotropic and osteotropic effect but no effect in the uterus or the mammary gland. Therefore, tectorigenin may be in the future a clinically useful selective estrogen receptor modulator.

Placenta (9)

http://www.sciencedirect.com/science/article/B6WPD-4C4VXX0-3/2/a334fae5de9a516939c65f0e70fcd224

Placenta growth factor (PIGF) is a homodimeric glycoprotein, 46-50 kDa in size, belonging to the vascular endothelial growth factor (VEGF) sub-family. It exists as two isoforms, PIGF-1 and -2, the latter having a heparin-binding domain. Like VEGF, it is a potent angiogenic factor; however, PIGF homodimers interact with the VEGF receptor Flt-1 (fms-like tyrosine kinase), but not with the kinase domain-containing region (KDR). Since PIGF is made by the human placenta and extravillous trophoblast (EVT) cells of the human placenta express Flt-1 in situ, these cells may be responsive to PIGF. Therefore, this study examined whether first trimester EVT cells propagated in vitro expressed the mRNA or the protein of Flt-1 and PIGF, and whether exogenous PIGF-1 had any effect on EVT cell proliferation, migration or invasiveness. Immunocytochemical and RT-PCR analyses revealed that both normal and SV40 Tag-immortalized EVT cells expressed the protein and mRNA for Flt-1, but not for P1GF-1 or -2. Exogenous PIGF-1 stimulated proliferation (measured by 3H-thymidine uptake) of normal EVT cells in a concentration-dependent manner, but only in the presence of excess heparan sulphate proteoglycans (HSPGs). These results raise two possibilities: that exogenous PIGF-1 (in spite of having a low affinity for heparin) was sequestered away from its receptor because of binding to heparan sulphate proteoglycans on the EVT cell surface or the ECM, or that HSPGs could modify the interaction between Flt-1 and PIGF. PIGF-1, in the presence or absence of HSPGs, however, had no effect on EVT migration or invasiveness, when measured with a transwell invasion (in the presence of Matrigel(TM)) or migration (in the absence of Matrigel(TM)) assay. These findings place PIGF amongst a large group of growth factors that promote EVT cell proliferation without influencing their migratory or invasive behaviours, and suggest that PIGF-Flt-1 interactions may be regulated by HSPGs in situ.


http://www.sciencedirect.com/science/article/B6WPD-4B5JNC8-3/2/5a58bbfa60a3d2dd7de9ae7d43856465

Pre-eclampsia is a multisystem disorder of pregnancy associated with elevated blood pressure, proteinuria, and complex biochemical disturbances. The mammalian homologue of the glial cells missing (GCM) gene, GCM1, is selectively expressed in the placenta. GCM1 expression has been shown to affect placental branching and vasculogenesis, abnormalities of which may result in the development of pre-eclampsia. In this study immunohistochemistry, Western blot, and quantitative real-time PCR were used to investigate GCM1 expression at different gestational ages and in pre-eclampsia. Of 36 placentae without pre-eclampsia (ranged from 5-40 weeks of gestation), the level of GCM1 expression was relatively constant before late third trimester. The immunoreactivity of GCM1 protein and the level of GCM1 mRNA were not significantly different during normal pregnancy until 37 weeks of gestation, when the level of GCM1 expression was reduced significantly. Furthermore, significant reductions in GCM1 protein and mRNA were observed in pre-eclamptic placentae compared with gestational age-matched controls. Our results suggest that GCM1 is a distinct transcription factor involved in placental disease and altered expression of the GCM1 gene may contribute to the etiology of pre-eclampsia.

http://www.sciencedirect.com/science/article/B6WPD-4C0CMCM-1T/2/cb47b7e840978951c0e7ac87c266d6a2

A comparative study of thrombomodulin (TM), a potent natural anticoagulant, was performed in first trimester and term human placentae. Immunoreactive TM was observed on fetal vascular endothelium and syncytiotrophoblast at both gestational ages. Staining was stronger in term than in early placentae, particularly along the microvillous apical membrane of the syncytiotrophoblast. Similarly, a higher level of TM mRNA was detected by RT-PCR (PPP<0.01) in first trimester and term microvilli, respectively. The modulation of biologically active TM at the syncytial membrane exposed to maternal blood according to the length of gestation suggests that TM may be involved both in maternal haemostasis within the intervillous spaces, and also in the trophoblast differentiation process.


http://www.sciencedirect.com/science/article/B6WPD-4BYRSNY-2/2/5720aa2282969c4d7e0f1e47d7eb72859

In this study, we performed the differential display technique to identify genes specifically expressed in human choriocarcinoma cell lines (JEG-3, JAR and BeWo) and normal placental term cells. Few differences were found among the expression profiles of the three choriocarcinoma cell lines and most of the differentially expressed genes were detected in normal term placenta. A total of 36 cDNA fragments were isolated and analysed. Of these, 19 sequences corresponded to regions in the human genome coding for potential novel genes. We confirmed by RT-PCR, the placental mRNA expression of three selected new human genes, on chromosomes 16q12, 9q32 and 6q22. The other 17 sequences showed high similarity to known human genes (like PSG3, FN1, PAI-2). Interestingly, the functions of five known proteins (from genes ILK, TRA-1, HERPUD1, UBA-2, and TRAP240) have not yet been well characterized in placenta tissue. In addition, new alternative spliced mRNAs were detected for ILK, TRAP240 and PLAC3 genes. The differential expression of the PAI-2 gene among the choriocarcinoma cell lines was also confirmed. The genes identified in this analysis will be of interest for future studies regarding both a better understanding of the biology of the trophoblast cell and the formation of placental tumors.


http://www.sciencedirect.com/science/article/B6WPD-4C0V5NX-3/2/9905f24382af39aa8b1af148e6bc0d36

This study determines whether genetic variability in the gene encoding factor V contributes to differences in susceptibility to placental abruption. Allele and genotype frequencies of three single nucleotide polymorphisms (SNPs) in the factor V gene leading to nonsynonymous changes (M385T in exon 8, and R485K and R506Q [Leiden mutation] in exon 10) were studied in 116 Caucasian women with placental abruption and 112 healthy controls. Single-point analysis was expanded to haplotype analysis and haplotype frequencies were estimated using an expectation-maximisation (EM) algorithm. Comparison of single-point allele and genotype distributions of
SNPs in exon 8 and exon 10 of the factor V gene revealed statistically significant differences in M385T allele (P=0.021) and genotype (P=0.013) frequencies between the patients and the control subjects. The C allele of SNP M385T was significantly less frequent among the patients (7%) vs. the control subjects (13%), at an odds ratio of 0.48 (95% CI 0.25-0.91). Allele and genotype differences between the patients and control subjects as regards R485K and Leiden mutation were not significant. In haplotype estimation analysis, there was a significantly lower frequency of haplotype T-R-R encoding the T385-R485-R506 variant in the group with placental abruption vs. the control group (P=0.038) at an odds ratio of 0.519 (95% CI 0.272-0.987). We conclude that T385 is less frequent among the patient group than in the control group. The M385T variant in the factor V gene other than the Leiden mutation may play a role in disease susceptibility.


Smoking during pregnancy causes low birth weight, premature delivery, neonatal morbidity, and mortality. Nicotine is a main pathogenic compound of cigarette smoke, and depresses active amino-acid uptake by human placental villi. It binds to the acetylcholine binding site of the [alpha]-subunits of nicotinic acetylcholine receptors (nAChR). Eight different neuronal nAChR [alpha]-subunits have been identified in mammals. Here, we investigated their localisation and distribution in the human and rat placenta by RT-PCR and immunofluorescence. The mRNAs of all [alpha]-subunits are expressed in the human and rat placenta. Immunohistochemically, subunits [alpha]2-5, [alpha]7, [alpha]9 and [alpha]10 are localised in different combinations in rat cytotrophoblast, human and rat syncytiotrophoblast, vascular smooth muscle cells, endothelial cells, Hofbauer cells, human amnion epithelium and rat visceral yolk sac epithelium. Thus, all human and rat placental cell types exhibit receptor subunits with binding sites for the endogenous ligand ACh and nicotine. ACh is suggested to be an important placental signalling molecule that, through stimulation of nAChR, controls the uptake of nutrients, blood flow and fluid volume in placental vessels, and the vascularisation during placental development. Chronic stimulation of nAChR by nicotine might result in unbalanced receptor activation or functional desensitisation followed by the known pathological effects of smoking.


http://www.sciencedirect.com/science/article/B6WPD-4B0X5C6-1/2/3d24cb904e4dfb76d8f4193fa9b67221

Transferrin (TF), a 76-80 kDa glycoprotein, is responsible for the transport of iron to cells within both the fetal and maternal systems, but it does not cross the multiple cell layer barrier of the placenta. Recent findings that both rat and human placental cells produce TF indicated that placental TF may function in some manner to transport or regulate iron passage across this barrier. However, placental production of TF was brought into question because the cell preparations used to identify TF were obtained using dispersed tissue and may have contained non-placental contaminating elements. In this study, cultures of phenotypically distinct cell types containing only placental cells were used to firmly establish whether or not TF is expressed, and if so to begin to identify the cell(s) associated with its synthesis. Utilizing RT-PCR, in situ hybridization, and Western blot analysis, we identified TF mRNA and protein in three trophoblast cell types, HRP-1 (rat), Rcho-1 (rat), and BeWo (human) cells. Additionally, TF mRNA and protein were found in Giant cells, the differentiated form of Rcho-1 cells. When taken together, these results demonstrate clearly that TF is expressed by both differentiated and non-differentiated...
placental cells, and when viewed in light of previous findings, strengthen the possibility that placental TF may be central to the passage of iron from the mother to the fetus during development.

http://www.sciencedirect.com/science/article/B6WPD-4DH2C1X-1/2/01c089d6ec31db17ab36832ff4a8e042

Serotonin (5-hydroxytryptamine, 5-HT) has diverse physiological functions and acts as a mitogen in a variety of cell types, including bovine placental cells. It exerts its mitogenic effect by interacting with a wide range of 5-HT receptor types. Previous studies have reported the presence of 5-HT2 binding sites in human placental trophoblastic cells, but this has never been confirmed at the molecular level. In this study, we demonstrated that the 5-HT2A receptor subtype is fully expressed in the human choriocarcinoma cell lines JEG-3 and BeWo as well as in normal human placental tissue. DNA sequencing has confirmed that the 5-HT2A receptor present in these cell lines and tissues is identical to the human 5-HT2A receptor found in platelets and in the cerebral cortex. This receptor was localized by immunofluorescence on the plasma membrane, in JEG-3 and BeWo cells. Furthermore, MTT proliferation assays revealed a positive effect of 5-HT on the proliferation of JEG-3 and BeWo cells. These results suggest that 5-HT constitutes a potent mitogen for neoplastic placental cells.

http://www.sciencedirect.com/science/article/B6WPD-4FBM1JM-1/2/e743b4ea80d808f6c2f6efa4fd55de2

The proteoglycan perlecan is involved in cell signaling, regulation of growth factor activity, and maintenance of basement membranes. This study aims to investigate the expression of perlecan during placental development and whether hyperglycemia of gestational diabetes mellitus induces the alteration of perlecan expression in placenta. Immunohistochemistry, immunoprecipitation/sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and quantitative real-time PCR were carried out to study the placental perlecan expression at different trimesters of pregnancies and in gestational diabetes mellitus. The perlecan protein was mainly immunolocalized in the trophoblast and vessel basement membranes with some staining in the villous stroma of placental villus. Perlecan was also found to co-localize with laminin and collagen IV in the basement membranes of placenta. The protein and mRNA levels of placental perlecan were significantly decreased as the gestational age increased. However, a significant increase in perlecan expression was observed in the third trimester placentas with gestational diabetes mellitus compared to the gestational age-matched controls. Furthermore, trophoblast cells cultured in a high glucose (30 mM) medium and a high osmotic pressure medium (5.6 mM glucose and 24.4 mM mannitol) showed increased perlecan expression compared to cells cultured in the low glucose (5.6 mM) regular medium. These alterations of perlecan expression may be associated with the structural changes of placenta during maturation. The metabolic effect of high glucose and high osmotic pressure of gestational diabetes mellitus may contribute to the increased perlecan expression of diabetic placentas.
PLANT CELL (12)


http://www.plantcell.org/cgi/content/abstract/15/12/3033

The tomato transcription factor Pti4, an ethylene-responsive factor (ERF), interacts physically with the disease resistance protein Pto and binds the GCC box cis element that is present in the promoters of many pathogenesis-related (PR) genes. We reported previously that Arabidopsis plants expressing Pti4 constitutively express several GCC box-containing PR genes and show reduced disease symptoms compared with wild-type plants after inoculation with Pseudomonas syringae pv tomato or Erysiphe orontii. To gain insight into how genome-wide gene expression is affected by Pti4, we used serial analysis of gene expression (SAGE) to compare transcripts in wild-type and Pti4-expressing Arabidopsis plants. SAGE provided quantitative measurements of >20,000 transcripts and identified the 50 most highly expressed genes in Arabidopsis vegetative tissues. Comparison of the profiles from wild-type and Pti4-expressing Arabidopsis plants revealed 78 differentially abundant transcripts encoding defense-related proteins, protein kinases, ribosomal proteins, transporters, and two transcription factors (TFs). Many of the genes identified were expressed differentially in wild-type Arabidopsis during infection by Pseudomonas syringae pv tomato, supporting a role for them in defense-related processes. Unexpectedly, the promoters of most Pti4-regulated genes did not have a GCC box. Chromatin immunoprecipitation experiments confirmed that Pti4 binds in vivo to promoters lacking this cis element. Potential binding sites for ERF, MYB, and GBF TFs were present in statistically significantly increased numbers in promoters regulated by Pti4. Thus, Pti4 appears to regulate gene expression directly by binding the GCC box and possibly a non-GCC box element and indirectly by either activating the expression of TF genes or interacting physically with other TFs.


http://www.plantcell.org/cgi/content/abstract/15/8/1833

Although calcium is a critical component in the signal transduction pathways that lead to stress gene expression in higher plants, little is known about the molecular mechanism underlying calcium function. It is believed that cellular calcium changes are perceived by sensor molecules, including calcium binding proteins. The calcineurin B-like (CBL) protein family represents a unique group of calcium sensors in plants. A member of the family, CBL1, is highly inducible by multiple stress signals, implicating CBL1 in stress response pathways. When the CBL1 protein level was increased in transgenic Arabidopsis plants, it altered the stress response pathways in these plants. Although drought-induced gene expression was enhanced, gene induction by cold was inhibited. In addition, CBL1-overexpressing plants showed enhanced tolerance to salt and drought but reduced tolerance to freezing. By contrast, cbl1 null mutant plants showed enhanced cold induction and reduced drought induction of stress genes. The mutant plants displayed less tolerance to salt and drought but enhanced tolerance to freezing. These studies suggest that CBL1 functions as a positive regulator of salt and drought responses and a negative regulator of cold response in plants.

http://www.plantcell.org/cgi/content/abstract/14/10/2495

Although it is well known that Tyr phosphatases play a critical role in signal transduction in animal cells, little is understood of the functional significance of Tyr phosphatases in higher plants. Here, we describe the functional analysis of an Arabidopsis gene (AtPTEN1) that encodes a Tyr phosphatase closely related to PTEN, a tumor suppressor in animals. The recombinant AtPTEN1 protein, like its homologs in animals, is an active phosphatase that dephosphorylates phosphotyrosine and phosphatidylinositol substrates. RNA gel blot analysis and examination of promoter-reporter constructs in transgenic Arabidopsis plants revealed that the AtPTEN1 gene is expressed exclusively in pollen grains during the late stage of development. Suppression of AtPTEN1 gene expression by RNA interference caused pollen cell death after mitosis. We conclude that AtPTEN1 is a pollen-specific phosphatase and is essential for pollen development.


http://www.plantcell.org/cgi/content/abstract/16/6/1446

The hydroxyl group in the 3-position of the phenylpropanoid compounds is introduced at the level of coumarate shikimate/quinate esters, whose synthesis implicates an acyltransferase activity. Specific antibodies raised against the recombinant tobacco (Nicotiana tabacum) acyltransferase revealed the accumulation of the enzyme in stem vascular tissues of tobacco, in accordance with a putative role in lignification. For functional analysis, the acyltransferase gene was silenced in Arabidopsis thaliana and N. benthamiana by RNA-mediated posttranscriptional gene silencing. In Arabidopsis, gene silencing resulted in a dwarf phenotype and changes in lignin composition as indicated by histochemical staining. An in-depth study of silenced N. benthamiana plants by immunological, histochemical, and chemical methods revealed the impact of acyltransferase silencing on soluble phenylpropanoids and lignin content and composition. In particular, a decrease in syringyl units and an increase in p-hydroxyphenyl units were recorded. Enzyme immunolocalization by confocal microscopy showed a correlation between enzyme accumulation levels and lignin composition in vascular cells. These results demonstrate the function of the acyltransferase in phenylpropanoid biosynthesis.


http://www.plantcell.org/cgi/content/abstract/15/2/411

Plants respond to environmental stress by activating "stress genes." The plant hormone abscisic acid (ABA) plays an important role in stress-responsive gene expression. Although Ca2+ serves as a common second messenger in signaling stress and ABA, little is known about the molecular basis of Ca2+ action in these pathways. Here, we show that CIPK3, a Ser/Thr protein kinase that associates with a calcineurin B-like calcium sensor, regulates ABA response during seed germination and ABA- and stress-induced gene expression in Arabidopsis. The expression of the CIPK3 gene itself is responsive to ABA and stress conditions, including cold, high salt, wounding, and drought. Disruption of CIPK3 altered the expression pattern of a number of stress gene
markers in response to ABA, cold, and high salt. However, drought-induced gene expression was not altered in the cipk3 mutant plants, suggesting that CIPK3 regulates select pathways in response to abiotic stress and ABA. These results identify CIPK3 as a molecular link between stress- and ABA-induced calcium signal and gene expression in plant cells. Because the cold signaling pathway is largely independent of endogenous ABA production, CIPK3 represents a cross-talk "node" between the ABA-dependent and ABA-independent pathways in stress responses.


http://www.plantcell.org/cgi/content/abstract/14/11/2813

Isolated mesophyll cells from Zinnia elegans are induced by auxin and cytokinin to form tracheary elements (TEs) in vitro with high synchrony. To reveal the changing patterns of gene expression during the 48 h of transdifferentiation from mesophyll to TE cell fate, we used a cDNA-amplified fragment length polymorphism approach to generate expression profiles of >30,000 cDNA fragments. Transcriptional changes of 652 cDNA fragments were observed, of which 304 have no previously described function or sequence identity. Sixty-eight genes were upregulated within 30 min of induction and represent key candidates for the processes that underlie the early stages of commitment and differentiation to a TE cell fate.


http://www.plantcell.org/cgi/content/abstract/17/3/705

The functions of the vast majority of genes encoding R2R3 MYB domain proteins remain unknown. The closely related MYB33 and MYB65 genes of Arabidopsis thaliana have high sequence similarity to the barley (Hordeum vulgare) GAMYB gene. T-DNA insertional mutants were isolated for both genes, and a myb33 myb65 double mutant was defective in anther development. In myb33 myb65 anthers, the tapetum undergoes hypertrophy at the pollen mother cell stage, resulting in premeiotic abortion of pollen development. However, myb33 myb65 sterility was conditional, where fertility increased both under higher light or lower temperature conditions. Thus, MYB33/MYB65 facilitate, but are not essential for, anther development. Neither single mutant displayed a phenotype, implying that MYB33 and MYB65 are functionally redundant. Consistent with functional redundancy, promoter-(beta)-glucuronidase (GUS) fusions of MYB33 and MYB65 gave identical expression patterns in flowers (sepals, style, receptacle, anther filaments, and connective but not in anthers themselves), shoot apices, and root tips. By contrast, expression of a MYB33:GUS translational fusion in flowers was solely in young anthers (consistent with the male sterile phenotype), and no staining was seen in shoot meristems or root tips. A microRNA target sequence is present in the MYB genes, and mutating this sequence in MYB33:GUS fusion results in an expanded expression pattern, in tissues similar to that observed in the promoter-GUS lines, implying that the microRNA target sequence is restricting MYB33 expression. Arabidopsis transformed with MYB33 containing the mutated microRNA target had dramatic pleiotropic developmental defects, suggesting that restricting MYB33 expression, especially in the shoot apices, is essential for proper plant development.

http://www.plantcell.org/cgi/content/abstract/15/8/1795

We investigated the effects of human selection for yellow endosperm color, representing increased carotenoid content, on two maize genes, the Y1 phytoene synthase and PSY2, a putative second phytoene synthase. Multiple polymorphic sites were identified at Y1 and PSY2 in 75 white and yellow maize inbred lines. Many polymorphic sites showed strong association with the endosperm color phenotype at Y1, but no detectable association was found at PSY2. Nucleotide diversity was equivalent for whites and yellows at PSY2 but was 19-fold less in yellows than in whites at Y1, consistent with the white ancestral state of the gene. The strong sequence haplotype conservation within yellows at Y1 and a significant, negative Tajima's D both verified positive selection for yellow endosperm. We propose that two independent gain-of-function events associated with insertions into the promoter of the Y1 gene and upregulation of expression in endosperm have been incorporated into yellow maize.


http://www.plantcell.org/cgi/content/abstract/14/6/1329

A characteristic plant response to microbial attack is the production of endo-(beta)-1,3-glucanases, which are thought to play an important role in plant defense, either directly, through the degradation of (beta)-1,3/1,6-glucans in the pathogen cell wall, or indirectly, by releasing oligosaccharide elicitors that induce additional plant defenses. We report the sequencing and characterization of a class of proteins, termed glucanase inhibitor proteins (GIPs), that are secreted by the oomycete Phytophthora sojae, a pathogen of soybean, and that specifically inhibit the endoglucanase activity of their plant host. GIPs are homologous with the trypsin class of Ser proteases but are proteolytically nonfunctional because one or more residues of the essential catalytic triad is absent. However, specific structural features are conserved that are characteristic of protein-protein interactions, suggesting a mechanism of action that has not been described previously in plant pathogen studies. We also report the identification of two soybean endoglucanases: EGaseA, which acts as a high-affinity ligand for GIP1; and EGaseB, with which GIP1 does not show any association. In vitro, GIP1 inhibits the EGaseA-mediated release of elicitor-active glucan oligosaccharides from P. sojae cell walls. Furthermore, GIPs and soybean endoglucanases interact in vivo during pathogenesis in soybean roots. GIPs represent a novel counterdefensive weapon used by plant pathogens to suppress a plant defense response and potentially function as important pathogenicity determinants.


http://www.plantcell.org/cgi/content/abstract/17/3/746

Carotenoids and carotenoid cleavage products play an important and integral role in plant development. The Decreased apical dominance1 (Dad1)/PhCCD8 gene of petunia (Petunia hybrida) encodes a hypothetical carotenoid cleavage dioxygenase (CCD) and ortholog of the MORE AXILLARY GROWTH4 (MAX4)/AtCCD8 gene. The dad1-1 mutant allele was inactivated
by insertion of an unusual transposon (Dad-one transposon), and the dad1-3 allele is a revertant allele of dad1-1. Consistent with its role in producing a graft-transmissible compound that can alter branching, the Dad1/PhCCD8 gene is expressed in root and shoot tissue. This expression is upregulated in the stems of the dad1-1, dad2, and dad3 increased branching mutants, indicating feedback regulation of the gene in this tissue. However, this feedback regulation does not affect the root expression of Dad1/PhCCD8. Overexpression of Dad1/PhCCD8 in the dad1-1 mutant complemented the mutant phenotype, and RNA interference in the wild type resulted in an increased branching phenotype. Other differences in phenotype associated with the loss of Dad1/PhCCD8 function included altered timing of axillary meristem development, delayed leaf senescence, smaller flowers, reduced internode length, and reduced root growth. These data indicate that the substrate(s) and/or product(s) of the Dad1/PhCCD8 enzyme are mobile signal molecules with diverse roles in plant development.


http://www.plantcell.org/cgi/content/abstract/16/4/819

Chalcone synthase, a key regulatory enzyme in the flavonoid pathway, constitutes an eight-member gene family in Glycine max (soybean). Three of the chalcone synthase (CHS) gene family members are arranged as inverted repeats in a 10-kb region, corresponding to the I locus (inhibitor). Spontaneous mutations of a dominant allele (I or ii) to a recessive allele (i) have been shown to delete promoter sequences, paradoxically increasing total CHS transcript levels and resulting in black seed coats. However, it is not known which of the gene family members contribute toward pigmentation and how this locus affects CHS expression in other tissues. We investigated the unusual nature of the I locus using four pairs of isogenic lines differing with respect to alleles of the I locus. RNA gel blots using a generic open reading frame CHS probe detected similar CHS transcript levels in stems, roots, leaves, young pods, and cotyledons of the yellow and black isolines but not in the seed coats, which is consistent with the dominant I and ii alleles mediating CHS gene silencing in a tissue-specific manner. Using real-time RT-PCR, a variable pattern of expression of CHS genes in different tissues was demonstrated. However, increase in pigmentation in the black seed coats was associated with release of the silencing effect specifically on CHS7/CHS8, which occurred at all stages of seed coat development. These expression changes were linked to structural changes taking place at the I locus, shown to encompass a much wider region of at least 27 kb, comprising two identical 10.91-kb stretches of CHS gene duplications. The suppressive effect of this 27-kb I locus in a specific tissue of the G. max plant represents a unique endogenous gene silencing mechanism.


http://www.plantcell.org/cgi/content/abstract/17/5/1559

Miniature inverted repeat transposable elements (MITEs) are thought to be a driving force for genome evolution. Although numerous MITEs are found associated with genes, little is known about their function in gene regulation. Whereas the rice ubiquitin2 (rubq2) promoter in rice (Oryza sativa) line IR24 contains two nested MITEs (Kiddo and MDM1), that in line T309 has lost Kiddo, providing an opportunity to understand the role of MITEs in promoter function. No difference in endogenous rubq2 transcript levels between T309 and IR24 was evident using RT-PCR. However, promoter analysis using both transient and stably transformed calli revealed that Kiddo contributed some 20% of the total expression. Bisulfite genomic sequencing of the rubq2 promoters revealed specific DNA methylation at both symmetric and asymmetric cytosine
residues on the MITE sequences, possibly induced by low levels of homologous transcripts. When methylation of the MITEs was blocked by 5-azacytidine treatment, a threefold increase in the endogenous rubq2 transcript level was detected in IR24 compared with that in T309. Together with the observed MITE methylation pattern, the detection of low levels of transcripts, but not small RNAs, corresponding to Kiddo and MDM1 suggested that RNA-dependent DNA methylation is induced by MITE transcripts. We conclude that, although Kiddo enhances transcription from the rubq2 promoter, this effect is mitigated by sequence-specific epigenetic modification.

**Plant Cell Physiol.** (9)


http://pcp.oupjournals.org/cgi/content/abstract/pci080v1

Silene latifolia is a dioecious plant in which sex is determined by X and Y chromosomes. Expression of the B-function gene SLM2, an ortholog of PISTILLATA (PI) in Arabidopsis, was examined by in situ hybridization. SLM2 was not expressed in suppressed stamens of female flowers, but was expressed in developing stamens of smut-infected female flowers. These results indicate that the control of SLM2 is independent of the presence of the Y chromosome. Smut-infected females provide a useful system for clarifying the relationship between the B-function gene and the sex determination factor.


http://pcp.oupjournals.org/cgi/content/abstract/45/6/795

We analyzed cell division patterns during the differentiation of unisexual flowers of the dioecious plant Silene latifolia using in situ hybridization with histone H4 and cyclin A1 genes. The gene expression patterns indicated that the activation of cell divisions in whorls 3 and 4 was reversed in young male and female flower buds. During maturation of flower buds, a remarkable reduction in cell division activity occurred in the male gynoecium primordium and female stamen primordia. Our analyses showed that differential activation and reduction of cell division strongly correlated with sex-specific promotion and cessation in the sex differentiation of unisexual flowers.


http://pcp.oupjournals.org/cgi/content/abstract/44/2/212

To understand a physiological role of an abundant 34-kDa periplasmic protein in the denitrifying
phototroph Rhodobacter sphaeroides f. sp. denitrificans grown in a medium containing malate as the carbon source, the gene for the protein was isolated. The deduced amino acid sequence of the protein had a sequence similarity of 66.2% to that of PstS from Sinorhizobium meliloti. The downstream sequence of the Rhodobacter pstS contained five genes similar to pstCAB and phoUB, and its upstream sequence contained a putative regulatory sequence that is analogous to the Pho box involved in phosphate-limitation-induced gene expression in Escherichia coli. Both the amount of the PstS and the pstS promoter-driven expression of lacZ activity increased about two-fold in response to phosphate limitation. This is the first isolation of pst genes encoding proteins of an ABC phosphate transporter system from phototrophic bacteria.


http://pcp.oupjournals.org/cgi/content/abstract/43/5/563

Fluorescent differential display (FDD) has been used to screen for cDNAs that are differentially up-regulated in male flowers of the dioecious plant Silene latifolia in which an X/Y chromosome system of sex determination operates. To adapt FDD to the cloning of large numbers of differential cDNAs, a novel method of confirming the differential expression of these has been devised. FDD gels were Southern electro-blotted and probed with mixtures of individual cDNA clones derived from different FDD product ligation reactions. These Southern blots were then stripped and re-probed with further mixtures of individual cloned FDD products to identify the maximum number of recombinant clones carrying the true differential amplification products. Of 135 differential bands identified by FDD, 56 differential amplification products were confirmed; these represent 23 unique differentially expressed genes as determined by virtual Northern analysis and two genes expressed at or below the level of detection by virtual Northern analysis. These two low expressed genes show bands of hybridization on genomic Southern blots that are specific to male plants, indicating that they are derived from, or closely related to, Y chromosome genes.


http://pcp.oupjournals.org/cgi/content/abstract/44/7/726

To study the regulation of ammonium uptake into rice roots, three ammonium transporter genes (OsAMT1;1, 1;2 and 1;3; Oryza sativa ammonium transporter) were isolated and examined. OsAMT1s belong to AMT1 family, containing 11 putative transmembrane-spanning domains. Southern blot analysis and screening of the rice genome database confirmed that with OsAMT1;1-1;3 the complete AMT1 family of rice had been isolated. Heterologous expression of OsAMT1s in the yeast Saccharomyces cerevisiae mutant 31019b showed that all three OsAMT1s exhibit ammonium transport activity. Northern blot analysis showed a distinct expression pattern for the three genes; more constitutive expression in shoots and roots for OsAMT1;1, root-specific and ammonium-inducible expression for OsAMT1;2, and root-specific and nitrogen-derepressible expression for OsAMT1;3. In situ mRNA detection revealed that OsAMT1;2 is expressed in the central cylinder and cell surface of root tips. This gene expression analysis revealed a distinct nitrogen-dependent regulation for AMTs in rice, differing from that in tomato or Arabidopsis.


http://pcp.oupjournals.org/cgi/content/abstract/44/12/1396

The three members of the rice OsAMT1 gene family of ammonium transporters show distinct expression patterns; constitutive and ammonium-promoted expression in shoots and roots for OsAMT1;1; root-specific and ammonium-inducible expression for OsAMT1;2; root-specific and nitrogen-repressible expression for OsAMT1;3 [Sonoda et al. (2003), Plant Cell Physiol. 44: 726]. To clarify the feedback mechanisms, and to identify regulatory factors of the OsAMT1 genes, the accumulation of the three mRNAs and its dependence on endogenous nitrogen compounds (as quantified by capillary electrophoresis) was studied. Ammonium application to roots following a period of nitrogen starvation induced accumulation of OsAMT1;1 and OsAMT1;2 mRNA, but a decrease of OsAMT1;3 mRNA levels. The expression patterns of the three genes showed good correlation (positive in OsAMT1;1 and OsAMT1;2, negative in OsAMT1;3) with the root tissue contents of glutamine but not of ammonium. The ammonium effects on OsAMT1 expression were prevented by methionine sulfoximine, an inhibitor of glutamine synthetase. Moreover, glutamine had the same effect on transcriptional regulation of OsAMT1 genes as ammonium, indicating that glutamine rather than ammonium controls the expression of ammonium transporter genes in rice. These results imply that rice possesses unique mechanisms of adaptation to variable nitrogen sources in the soil.


http://pcp.oupjournals.org/cgi/content/abstract/43/11/1323

The tapping panel dryness (TPD) syndrome of rubber is characterized by the reduction or ultimately total cessation of latex flow upon tapping, due to physiological disorders in the bark tissue. The protein pattern in the cytoplasm from healthy and TPD tree latex cells was compared by electrophoresis. Two polypeptides (P15 and P22) of 15 and 22 kDa, respectively, were found to accumulate in the cytosol of the TPD-affected trees, whereas a 29 kDa polypeptide (P29) appeared de novo. P15 and P22 were identified as REF (Hev b1) and SRPP (Hev b3), respectively, two proteins proposed to be involved in rubber biosynthesis. P29 appeared to be a new member of the patatin-like protein family. Specific molecular probes were designed for a detailed characterization of REF and SRPP gene expression and RFLP mapping. This allowed the demonstration that REF and SRPP display very similar expression profiles. They are highly over-expressed by the tapping-induced metabolic activation, although not by wounding per se, or ethylene or ABA. In addition to this similarity in gene expression, they were found to share one common locus in the genome. No significant difference in REF and SRPP gene expression was observed between healthy and TPD trees, indicating that their TPD-related accumulation in the cytosol was not transcriptionally regulated. Western blot analysis demonstrated that osmotic lysis of the sedimentable organelles (lutoids) in vitro caused the release of REF and SRPP from the rubber particle membrane into the cytosol. A mechanism of cellular delocalization as a consequence of the lutoids instability is proposed to explain REF and SRPP accumulation in the cytosol of TPD trees.


http://pcp.oupjournals.org/cgi/content/abstract/45/3/333
Multiple targeted gene replacements are often required for functional analyses of cyanobacterial genomes. For this purpose, we previously devised a simple genetic method, termed rps12-mediated gene replacement, in a cyanobacterium Synechococcus elongatus PCC 7942 for construction of mutants free from drug resistance markers. Here, we improved the method by employing a heterologous rps12 gene encoding a ribosomal protein S12 from Synechocystis sp. PCC 6803. Dominant streptomycin-sensitive phenotype of the Synechocystis rps12 gene was manifested only when it was expressed under the strong promoter of psbAI gene in S. elongatus PCC 7942 bearing a streptomycin-resistant rps12 allele. Transformation of the rps12 heteroallelic strains with non-replicating template plasmids permitted the selection of recombinants with gene replacement at frequencies up to 50% among streptomycin-resistant progeny.


http://pcp.oupjournals.org/cgi/content/abstract/45/12/1863

We developed seven Q-chromosome-specific DNA markers in Nicotiana tabacum by random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis using two hybrid lines, and we were able to identify tobacco monosomic plants among F1 progeny derived from the cross N. tabacum Haplo-Q x N. tabacum cv. Samsun NN using Q-chromosome-specific DNA markers. Based on the results, we discuss the roles of the Q chromosome in embryo sac development and embryogenesis. Here, we propose a new method for identifying DNA markers for a particular chromosome in the genus Nicotiana.


http://www.plantphysiol.org/cgi/content/abstract/131/4/1518

Substantial symplastic continuity appears to exist between companion cells (CCs) and sieve elements of the phloem, which suggests that small solutes within the CC are subject to indiscriminate long-distance transport via the translocation stream. To test this hypothesis, the distributions of exotic and endogenous solutes synthesized in the CCs of minor veins were studied. Octopine, a charged molecule derived from arginine and pyruvate, was efficiently transported through the phloem but was also transferred in substantial amounts to the apoplast, and presumably other non-phloem compartments. The disaccharide galactinol also accumulated in non-phloem compartments, but long-distance transport was limited. Conversely, sucrose, raffinose, and especially stachyose demonstrated reduced accumulation and efficient transport out of the leaf. We conclude that small metabolites in the cytosol of CCs do enter the translocation stream indiscriminately but are also subject to distributive forces, such as nonselective and carrier-mediated membrane transport and symplastic dispersal, that may effectively clear a compound from the phloem or retain it for long-distance transport. A model is proposed in which the transport of oligosaccharides is an adaptive strategy to improve
photoassimilate retention, and consequently translocation efficiency, in the phloem.


http://www.plantphysiol.org/cgi/content/abstract/131/3/1496

Arbuscular mycorrhizal (AM) fungi take up photosynthetically fixed carbon from plant roots and translocate it to their external mycelium. Previous experiments have shown that fungal lipid synthesized from carbohydrate in the root is one form of exported carbon. In this study, an analysis of the labeling in storage and structural carbohydrates after 13C1 glucose was provided to AM roots shows that this is not the only pathway for the flow of carbon from the intraradical to the extraradical mycelium (ERM). Labeling patterns in glycogen, chitin, and trehalose during the development of the symbiosis are consistent with a significant flux of exported glycogen. The identification, among expressed genes, of putative sequences for glycogen synthase, glycogen branching enzyme, chitin synthase, and for the first enzyme in chitin synthesis (glutamine fructose-6-phosphate aminotransferase) is reported. The results of quantifying glycogen synthase gene expression within mycorrhizal roots, germinating spores, and ERM are consistent with labeling observations using 13C-labeled acetate and glycerol, both of which indicate that glycogen is synthesized by the fungus in germinating spores and during symbiosis. Implications of the labeling analyses and gene sequences for the regulation of carbohydrate metabolism are discussed, and a 4-fold role for glycogen in the AM symbiosis is proposed: sequestration of hexose taken from the host, long-term storage in spores, translocation from intraradical mycelium to ERM, and buffering of intracellular hexose levels throughout the life cycle.


http://www.plantphysiol.org/cgi/content/abstract/128/1/108

The arbuscular mycorrhizal (AM) symbiosis is responsible for huge fluxes of photosynthetically fixed carbon from plants to the soil. Carbon is transferred from the plant to the fungus as hexose, but the main form of carbon stored by the mycobiont at all stages of its life cycle is triacylglycerol. Previous isotopic labeling experiments showed that the fungus exports this storage lipid from the intraradical mycelium (IRM) to the extraradical mycelium (ERM). Here, in vivo multiphoton microscopy was used to observe the movement of lipid bodies through the fungal colony and to determine their sizes, distribution, and velocities. The distribution of lipid bodies along fungal hyphae suggests that they are progressively consumed as they move toward growing tips. We report the isolation and measurements of expression of an AM fungal expressed sequence tag that encodes a putative acyl-coenzyme A dehydrogenase; its deduced amino acid sequence suggests that it may function in the anabolic flux of carbon from lipid to carbohydrate. Time-lapse image sequences show lipid bodies moving in both directions along hyphae and nuclear magnetic resonance analysis of labeling patterns after supplying 13C-labeled glycerol to either extraradical hyphae or colonized roots shows that there is indeed significant bidirectional translocation between IRM and ERM. We conclude that large amounts of lipid are translocated within the AM fungal colony and that, whereas net movement is from the IRM to the ERM, there is also substantial recirculation throughout the fungus.

We identified a dwarf transgenic hybrid poplar (Populus tremula x Populus alba) after screening of 627 independent activation-tagged transgenic lines in tissue culture, greenhouse, and field environments. The cause of the phenotype was a hyperactivated gene encoding GA 2-oxidase (GA2ox), the major gibberellin (GA) catabolic enzyme in plants. The mutation resulted from insertion of a strong transcriptional enhancer near the transcription start site. Overexpression of the poplar GA2ox gene (PtaGA2ox1) caused hyperaccumulation of mRNA transcripts, quantitative shifts in the spectrum of GAs, and similarity in phenotype to transgenic poplars that overexpress a bean (Phaseolus coccineus) GA2ox gene. The poplar PtaGA2ox1 sequence was most closely related to PsGA2ox2 from pea (Pisum sativum) and two poorly known GA2oxs from Arabidopsis (AtGA2ox4 and AtGA2ox5). The dwarf phenotype was reversible through gibberellic acid application to the shoot apex. Transgenic approaches to producing semidwarf trees for use in arboriculture, horticulture, and forestry could have significant economic and environmental benefits, including altered fiber and fruit production, greater ease of management, and reduced risk of spread in wild populations.


Although cold and drought adaptation in cereals and other plants involve the induction of a large number of genes, inheritance studies in Triticeae (wheat [Triticum aestivum], barley [Hordeum vulgare], and rye [Secale cereale]) have revealed only a few major loci for frost or drought tolerance that are consistent across multiple genetic backgrounds and environments. One might imagine that these loci could encode highly conserved regulatory factors that have global effects on gene expression; therefore, genes encoding central regulators identified in other plants might be orthologs of these Triticeae stress tolerance genes. The CBF/DREB1 regulators, identified originally in Arabidopsis as key components of cold and drought regulation, merit this consideration. We constructed barley cDNA libraries, screened these libraries and a barley bacterial artificial chromosome library using rice (Oryza sativa) and barley Cbf probes, found orthologs of Arabidopsis CBF/DREB1 genes, and examined the expression and genetic map location of the barley Cbf3 gene, HvCbf3. HvCbf3 was induced by a chilling treatment. HvCbf3 is located on barley chromosome 5H between markers WG364b and saflp58 on the barley cv Dicktoo x barley cv Morex genetic linkage map. This position is some 40 to 50 cM proximal to the winter hardiness quantitative trait locus that includes the Vrn-1H gene, but may coincide with the wheat 5A Rcg1 locus, which governs the threshold temperature at which cor genes are induced. From this, it remains possible that HvCbf3 is the basis of a minor quantitative trait locus in some genetic backgrounds, though that possibility remains to be thoroughly explored.

have characterized the full complement of pgip genes in the bean (Phaseolus vulgaris) genotype BAT93. This comprises four clustered members that span a 50-kb region and, based on their similarity, form two pairs (Pvpgip1/Pvpgip2 and Pvpgip3/Pvpgip4). Characterization of the encoded products revealed both partial redundancy and subfunctionalization against fungal-derived PGs. Notably, the pair PvPGIP3/PvPGIP4 also inhibited PGs of two mirid bugs (Lygus rugulipennis and Adelphocoris lineolatus). Characterization of Pvpgip genes of Pinto bean showed variations limited to single synonymous substitutions or small deletions. A three-amino acid deletion encompassing a residue previously identified as crucial for recognition of PG of Fusarium moniliforme was responsible for the inability of BAT93 PvPGIP2 to inhibit this enzyme.

Consistent with the large variations observed in the promoter sequences, reverse transcription-PCR expression analysis revealed that the different family members differentially respond to elicitors, wounding, and salicylic acid. We conclude that both biochemical and regulatory redundancy and subfunctionalization of pgip genes are important for the adaptation of plants to pathogenic fungi and phytophagous insects.


http://www.plantphysiol.org/cgi/content/abstract/137/3/873

(-)-Menthone is the predominant monoterpene produced in the essential oil of maturing peppermint (Mentha x piperita) leaves during the filling of epidermal oil glands. This early biosynthetic process is followed by a second, later oil maturation program (approximately coincident with flower initiation) in which the C3-carbonyl of menthone is reduced to yield (-)-(3R)-menthol and (+)-(3S)-neomenthol by two distinct NADPH-dependent ketoreductases. An activity-based in situ screen, by expression in Escherichia coli of 23 putative redox enzymes from an immature peppermint oil gland expressed sequence tag library, was used to isolate a cDNA encoding the latter menthone:(+)-(3S)-neomenthol reductase. Reverse transcription-PCR amplification and RACE were used to acquire the former menthone:(-)-(3R)-menthol reductase directly from mRNA isolated from the oil gland secretory cells of mature leaves. The deduced amino acid sequences of these two reductases share 73% identity, provide no apparent subcellular targeting information, and predict inclusion in the short-chain dehydrogenase/reductase family of enzymes. The menthone:(+)-(3S)-neomenthol reductase cDNA encodes a 35,722-D protein, and the recombinant enzyme yields 94% (+)-(3S)-neomenthol and 6% (-)-(3R)-menthol from (-)-menthone as substrate, and 86% (+)-(3S)-isomenthol and 14% (+)-(3R)-neoisomenthol from (+)-isomenthone as substrate, has a pH optimum of 9.3, and Km values of 674 {micro}M, > 1 mM, and 10 {micro}M for menthone, isomenthone, and NADPH, respectively, with a kcat of 0.06 s-1. The recombinant menthone:(-)-(3R)-menthol reductase has a deduced size of 34,070 D and converts (-)-menthone to 95% (-)-(3R)-menthol and 5% (+)-(3S)-neomenthol, and (+)-isomenthone to 87% (+)-(3R)-neoisomenthol and 13% (+)-(3S)-isomenthol, displays optimum activity at neutral pH, and has Km values of 3.0 {micro}M, 41 {micro}M, and 0.12 {micro}M for menthone, isomenthone, and NADPH, respectively, with a kcat of 0.6 s-1. The respective activities of these menthone reductases account for all of the menthol isomers found in the essential oil of peppermint. Biotechnological exploitation of these genes could lead to improved production yields of (-)-menthol, the principal and characteristic flavor component of peppermint.


http://www.plantphysiol.org/cgi/content/abstract/131/2/610
R2R3 Myb genes are widely distributed in the higher plants and comprise one of the largest known families of regulatory proteins. Here, we provide an evolutionary framework that helps explain the origin of the plant-specific R2R3 Myb genes from widely distributed R1R2R3 Myb genes, through a series of well-established steps. To understand the routes of sequence divergence that followed Myb gene duplication, we supplemented the information available on recently duplicated maize (Zea mays) R2R3 Myb genes (C1/P11 and P1/P2) by cloning and characterizing ZmMyb-IF35 and ZmMyb-IF25. These two genes correspond to the recently expanded P-to-A group of maize R2R3 Myb genes. Although the origins of C1/P11 and ZmMyb-IF35/ZmMyb-IF25 are associated with the segmental allotetraploid origin of the maize genome, other gene duplication events also shaped the P-to-A clade. Our analyses indicate that some recently duplicated Myb gene pairs display substantial differences in the numbers of synonymous substitutions that have accumulated in the conserved MYB domain and the divergent C-terminal regions. Thus, differences in the accumulation of substitutions during evolution can explain in part the rapid divergence of C-terminal regions for these proteins in some cases. Contrary to previous studies, we show that the divergent C termini of these R2R3 MYB proteins are subject to purifying selection. Our results provide an in-depth analysis of the sequence divergence for some recently duplicated R2R3 Myb genes, yielding important information on general patterns of evolution for this large family of plant regulatory genes.


http://www.plantphysiol.org/cgi/content/abstract/131/3/1347

Raffinose family oligosaccharides (RFOs) have been implicated in mitigating the effects of environmental stresses on plants. In seeds, proposed roles for RFOs include protecting cellular integrity during desiccation and/or imbibition, extending longevity in the dehydrated state, and providing substrates for energy generation during germination. A gene encoding galactinol synthase (GOLS), the first committed enzyme in the biosynthesis of RFOs, was cloned from tomato (Lycopersicon esculentum Mill. cv Moneymaker) seeds, and its expression was characterized in tomato seeds and seedlings. GOLS (LeGOLS-1) mRNA accumulated in developing tomato seeds concomitant with maximum dry weight deposition and the acquisition of desiccation tolerance. LeGOLS-1 mRNA was present in mature, desiccated seeds but declined within 8 h of imbibition in wild-type seeds. However, LeGOLS-1 mRNA accumulated again in imbibed seeds prevented from completing germination by dormancy or water deficit. Gibberellin-deficient (gib-1) seeds maintained LeGOLS-1 mRNA amounts after imbibition unless supplied with gibberellin, whereas abscisic acid (ABA) did not prevent the loss of LeGOLS-1 mRNA from wild-type seeds. The presence of LeGOLS-1 mRNA in ABA-deficient (sitiens) tomato seeds indicated that wild-type amounts of ABA are not necessary for its accumulation during seed development. In all cases, LeGOLS-1 mRNA was most prevalent in the radicle tip. LeGOLS-1 mRNA accumulation was induced by dehydration but not by cold in germinating seeds, whereas both stresses induced LeGOLS-1 mRNA accumulation in seedling leaves. The physiological implications of LeGOLS-1 expression patterns in seeds and leaves are discussed in light of the hypothesized role of RFOs in plant stress tolerance.


http://www.plantphysiol.org/cgi/content/abstract/131/2/684
To provide information on the roles of the different forms of ADP-glucose pyrophosphorylase (AGPase) in barley (Hordeum vulgare) endosperm and the nature of the genes encoding their subunits, a mutant of barley, Riso 16, lacking cytosolic AGPase activity in the endosperm was identified. The mutation specifically abolishes the small subunit of the cytosolic AGPase and is attributable to a large deletion within the coding region of a previously characterized small subunit gene that we have called Hv.AGP.S.1. The plastidial AGPase activity in the mutant is unaffected. This shows that the cytosolic and plastidial small subunits of AGPase are encoded by separate genes. We purified the plastidial AGPase protein and, using amino acid sequence information, we identified the novel small subunit gene that encodes this protein. Studies of the Riso 16 mutant revealed the following. First, the reduced starch content of the mutant showed that a cytosolic AGPase is required to achieve the normal rate of starch synthesis. Second, the mutant makes both A- and B-type starch granules, showing that the cytosolic AGPase is not necessary for the synthesis of these two granule types. Third, analysis of the phylogenetic relationships between the various small subunit proteins both within and between species, suggest that the cytosolic AGPase single small subunit gene probably evolved from a leaf single small subunit gene.

http://www.plantphysiol.org/cgi/content/abstract/135/1/357

With each passing year since the Chernobyl accident of 1986, more questions arise about the potential for organisms to adapt to radiation exposure. Often this is thought to be attributed to somatic and germline mutation rates in various organisms. We analyzed the adaptability of native Arabidopsis plants collected from areas with different levels of contamination around the Chernobyl nuclear power plant from 1986 to 1992. Notably, progeny of Chernobyl plants resisted higher concentrations of the mutagens Rose Bengal and methyl methane sulfonate. We analyzed the possible molecular mechanisms of their resistance to mutagens and found a more than 10-fold lower frequency of extrachromosomal homologous recombination, significant differences in the expression of radical scavenging (CAT1 and FSD3) and DNA-repair (RAD1 and RAD51-like) genes upon exposure to mutagens (Rose Bengal and x-rays), and a higher level of global genome methylation. This data suggests that adaptation to ionizing radiation is a complex process involving epigenetic regulation of gene expression and genome stabilization that improves plants' resistance to environmental mutagens.

http://www.plantphysiol.org/cgi/content/abstract/132/1/292

It has been proposed that fw2.2 encodes a negative fruit-growth regulator that underlies natural fruit-size variation in tomato (Lycopersicon spp.) via heterochronic allelic variation of fw2.2 expression, rather than by variation in the structural protein itself. To further test the negative regulator and the transcriptional control hypotheses, a gene dosage series was constructed, which produced a wider range of fw2.2 transcript accumulation than can be found in natural tomato populations. Fruit developmental analyses revealed that fw2.2 transcript levels were highly correlated (negatively) with fruit mass, supporting the negative regulator and transcriptional regulation hypotheses. Further, the effect of fw2.2 on fruit mass was mediated by repressing three- and two-dimensional cell division in placental and pericarp tissues, respectively. Finally, fw2.2 had little effect on fertility and seed size/number, indicating that fruit size effects of fw2.2 are due largely to expression in the maternal tissues of developing fruit and not mediated through
fertility or seed-setting-related processes.


http://www.plantphysiol.org/cgi/content/abstract/129/2/733

The joining of different genomes in allotetraploids played a major role in plant evolution, but the molecular implications of this event are poorly understood. In synthetic allotetraploids of Arabidopsis and Cardaminopsis arenosa, we previously demonstrated the occurrence of frequent gene silencing. To explore the involvement of epigenetic phenomena, we investigated the occurrence and effects of DNA methylation changes. Changes in DNA methylation patterns were more frequent in synthetic allotetraploids than in the parents. Treatment with 5-aza-2'-deoxycytidine, an inhibitor of DNA methyltransferase, resulted in the development of altered morphologies in the synthetic allotetraploids, but not in the parents. We profiled mRNAs in control and 5-aza-2'-deoxycytidine-treated parents and allotetraploids by amplified fragment length polymorphism-cDNA. We show that DNA demethylation induced and repressed two different transcriptomes. Our results are consistent with the hypothesis that synthetic allotetraploids have compromised mechanisms of epigenetic gene regulation.


http://www.plantphysiol.org/cgi/content/abstract/130/1/111

Functional analyses of a number of hydrolase gene promoters, induced by gibberellin (GA) in aleurone cells following germination, have identified a GA-responsive complex as a tripartite element containing a pyrimidine box motif 5'-CCTTTT-3'. We describe here that BPBF, a barley (Hordeum vulgare) transcription factor of the DOF (DNA-Binding with One Finger) class, previously shown to be an activator of reserve protein encoding genes during development, also has a role in the control of hydrolase genes following seed germination. Northern-blot, reverse transcriptase-polymerase chain reaction, and in situ hybridization analyses evidenced that the transcripts of the BPBF-encoding gene (Pbf), besides being present during endosperm development, are also expressed in aleurone cells of germinated seeds where they are induced by GA, an effect counteracted by abscisic acid. Electrophoretic mobility shift assays have shown that the BPBF protein binds specifically to the pyrimidine box motif in vitro within the different sequence contexts that naturally occur in the promoters of genes encoding a cathepsin B-like protease (Al21) and a low-isoelectric point [alpha]-amylase (Amy2/32b), both induced in the aleurone layers in response to GA. In transient expression experiments, BPBF repressed transcription of the Al21 promoter in GA-treated barley aleurone layers and reverted the GAMYB-mediated activation of this protease promoter.


http://www.plantphysiol.org/cgi/content/abstract/130/4/1614
A "gene-island" sequencing strategy has been developed that expedites the targeted acquisition of orthologous gene sequences from related species for comparative genome analysis. A 152-kb bacterial artificial chromosome (BAC) clone from sorghum (Sorghum bicolor) encoding phytochrome A (PHYA) was fully sequenced, revealing 16 open reading frames with a gene density similar to many regions of the rice (Oryza sativa) genome. The sequences of genes in the orthologous region of the maize (Zea mays) and rice genomes were obtained using the gene-island sequencing method. BAC clones containing the orthologous maize and rice PHYA genes were identified, sheared, subcloned, and probed with the sorghum PHYA-containing BAC DNA. Sequence analysis revealed that approximately 75% of the cross-hybridizing subclones contained sequences orthologous to those within the sorghum PHYA BAC and less than 25% contained repetitive and/or BAC vector DNA sequences. The complete sequence of four genes, including up to 1 kb of their promoter regions, was identified in the maize PHYA BAC. Nine orthologous gene sequences were identified in the rice PHYA BAC. Sequence comparison of the orthologous sorghum and maize genes aided in the identification of exons and conserved regulatory sequences flanking each open reading frame. Within genomic regions where micro-colinearity of genes is absolutely conserved, gene-island sequencing is a particularly useful tool for comparative analysis of genomes between related species.


http://www.plantphysiol.org/cgi/content/abstract/130/3/1298

Mutualistic fungal endophytes infect many grass species and often confer benefits to the hosts such as reduced herbivory by insects and animals. The physiological interactions between the endophytes and their hosts have not been well characterized. Fungal-secreted proteins are likely to be important components of the interaction. In the interaction between Poa ampla and the endophyte Neotyphodium sp., a fungal [beta]-1,6-glucanase is secreted into the apoplast, and activity of the enzyme is detectable in endophyte-infected plants. Sequence analysis indicates the [beta]-1,6-glucanase is homologous to enzymes secreted by the mycoparasitic fungi Trichoderma harzianum and Trichoderma virens. DNA gel-blot analysis indicated the [beta]-1,6-glucanase was encoded by a single gene. As a secreted protein, the [beta]-1,6-glucanase may have a nutritional role for the fungus. In culture, [beta]-1,6-glucanase activity was induced in the presence of [beta]-1,6-glucans. From RNA gel blots, similar [beta]-1,6-glucanases were expressed in tall fescue (Festuca arundinacea Schreb.) and Chewings fescue (Festuca rubra L. subsp. fallax [Thuill] Nyman) infected with the endophyte species Neotyphodium coenophialum and Epichloe festucae, respectively.


http://www.plantphysiol.org/cgi/content/abstract/129/4/1872

The effect of cadmium (Cd) on high-affinity sulfate transport of maize (Zea mays) roots was studied and related to the changes in the levels of sulfate and nonprotein thiols during Cd-induced phytochelatin (PC) biosynthesis. Ten micromolar CdCl2 in the nutrient solution induced a 100% increase in sulfate uptake by roots. This was not observed either for potassium or phosphate uptake, suggesting a specific effect of Cd2+ on sulfate transport. The higher sulfate uptake was not dependent on a change in the proton motive force that energizes it. In fact, in Cd-treated plants, the transmembrane electric potential difference of root cortical cells was only slightly more negative than in the controls, the external pH did not change, and the activity of the plasma membrane H+-ATPase did not increase. Kinetics analysis showed that in the range of the
high-affinity sulfate transport systems, 10 to 250 \( \mu \text{M} \), Cd exposure did not influence the Km value (about 20 \( \mu \text{M} \)), whereas it doubled the Vmax value with respect to the control. Northern-blot analysis showed that Cd-induced sulfate uptake was related to a higher level of mRNA encoding for a putative high-affinity sulfate transporter in roots. Cd-induced sulfate uptake was associated to both a decrease in the contents of sulfate and glutathione and synthesis of a large amount of PCs. These results suggest that Cd-induced sulfate uptake depends on a pretranslational regulation of the high-affinity sulfate transporter gene and that this response is necessary for sustaining the higher sulfur demand during PC biosynthesis.


http://www.plantphysiol.org/cgi/content/abstract/131/3/1294

Eurycoma longifolia Jack. is a treelet that grows in the forests of Southeast Asia and is widely used throughout the region because of its reported medicinal properties. Widespread harvesting of wild-grown trees has led to rapid thinning of natural populations, causing a potential decrease in genetic diversity among E. longifolia. Suitable genetic markers would be very useful for propagation and breeding programs to support conservation of this species, although no such markers currently exist. To meet this need, we have applied a genome complexity reduction strategy to identify a series of single nucleotide polymorphisms (SNPs) within the genomes of several E. longifolia accessions. We have found that the occurrence of these SNPs reflects the geographic origins of individual plants and can distinguish different natural populations. This work demonstrates the rapid development of molecular genetic markers in species for which little or no genomic sequence information is available. The SNP markers that we have developed in this study will also be useful for identifying genetic fingerprints that correlate with other properties of E. longifolia, such as high regenerability or the appearance of bioactive metabolites.


http://www.plantphysiol.org/cgi/content/abstract/131/3/1137

To understand further how pollination, seeds, auxin (4-chloroindole-3-acetic acid [4-Cl-IAA]), and gibberellins (GAs) regulate GA biosynthesis in pea (Pisum sativum) fruit, we studied expression of the gene PsGA3ox1 that codes for the enzyme that converts GA20 to biologically active GA1 using real-time reverse transcription-polymerase chain reaction analysis. PsGA3ox1 mRNA levels were minimally detectable in prepollinated pericarps and ovules ([-]2 d after anthesis [DAA]), increased dramatically after pollination (0 DAA), then decreased by 1 DAA. Seed PsGA3ox1 mRNA levels increased at 4 DAA and again 8 to 12 DAA, when seed development was rapid. Pericarp PsGA3ox1 mRNA levels peaked coincidentally with rapid pod diameter expansion (6-10 DAA) to accommodate the growing seeds. The effects of seeds and hormones on the expression of pericarp PsGA3ox1 were investigated over a 24-h treatment period. Pericarp PsGA3ox1 mRNA levels gradually increased from 2 to 3 DAA when seeds were present; however, when the seeds were removed, the pericarp transcript levels dramatically declined. When 2-DAA deseeded pericarps were treated with 4-Cl-IAA, PsGA3ox1 mRNA levels peaked 4 h after hormone treatment (270-fold increase), then decreased. PsGA3ox1 mRNA levels in deseeded pericarps treated with indole-3-acetic acid or GA3 were the same or lower than deseeded controls. These data show that PsGA3ox1 is expressed and developmentally regulated in pea pericarps and seeds. These data also show that pericarp PsGA3ox1 expression is hormonally regulated and suggest that the conversion of GA20 to GA1 occurs in the pericarp and is regulated by the
presence of seeds and 4-Cl-IAA for fruit growth.


http://www.plantphysiol.org/cgi/content/abstract/134/1/101

The dwarf ucu (ultracurvata) mutants of Arabidopsis display vegetative leaves that are spirally rolled downwards and show reduced expansion along the longitudinal axis. We have previously determined that the UCU1 gene encodes a SHAGGY/GSK3-like kinase that participates in the signaling pathways of auxins and brassinosteroids. Here, we describe four recessive alleles of the UCU2 gene, whose homozygotes display helical rotation of several organs in addition to other phenotypic traits shared with ucu1 mutants. Following a map-based strategy, we identified the UCU2 gene, which was found to encode a peptidyl-prolyl cis-trans-isomerase of the FK506-binding protein family, whose homologs in metazoa are involved in cell signaling and protein trafficking. Physiological and double mutant analyses suggest that UCU2 is required for growth and development and participates in auxin and brassinosteroid signaling.


http://www.plantphysiol.org/cgi/content/abstract/132/2/768

Xyloglucan (XyG) is a load-bearing primary wall component in dicotyledonous and non-graminaceous monocotyledonous plants. XyG fucosyltransferase (FUTase), encoded by the Arabidopsis gene AtFUT1, directs addition of fucose (Fuc) residues to terminal galactose residues on XyG side chains. Reverse transcription-polymerase chain reaction and analysis of promoter-(beta)-glucuronidase transgenic plants indicated highest expression of AtFUT1 in the upper portion of elongating inflorescence stems of Arabidopsis. XyG FUTase activity was highest in Golgi vesicles prepared from growing Arabidopsis tissues and low in those isolated from mature tissues. There was no discernible correlation between the Fuc contents of XyG oligosaccharides derived from different Arabidopsis organs and the level of AtFUT1 expression in the organs. Thus, organ-specific variations in AtFUT1 expression and enzyme activity probably reflect differential rates of cell wall biosynthesis, rather than differences in levels of XyG fucosylation. The effects of manipulating AtFUT1 expression were examined using an Arabidopsis mutant (atfut1) containing a T-DNA insertion in the AtFUT1 locus and transgenic plants with strong constitutive expression of AtFUT1. No Fuc was detected in XyG derived from leaves or roots of atfut1. Plants overexpressing AtFUT1 had higher XyG FUTase activity than wild-type plants, but the XyG oligosaccharides derived from the transgenic and wild-type plants contained comparable amounts of Fuc, indicating that suitable acceptor substrates are limiting. Galactosyl residues had slightly higher levels of O-acetylation in XyG from plants that overexpressed AtFUT1 than in XyG from wild-type plants. O-Acetylation of galactose residues was considerably reduced in Fuc-deficient mutants (atfut1, mur1, and mur2) that synthesize XyG containing little or no Fuc. These results suggest that fucosylated XyG is a suitable substrate for at least one O-acetyltransferase in Arabidopsis.

Carotenoids are thought to be the precursors of terpenoid volatile compounds that contribute to flavor and aroma. One such volatile, \( \beta \)-ionone, is important to fragrance in many flowers, including petunia (Petunia hybrida). However, little is known about the factors regulating its synthesis in vivo. The petunia genome contains a gene encoding a 9,10(9',10') carotenoid cleavage dioxygenase, PhCCD1. The PhCCD1 is 94% identical to LeCCD1A, an enzyme responsible for formation of \( \beta \)-ionone in tomato (Lycopersicon esculentum; Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ [2004] Plant J [in press]). Reduction of PhCCD1 transcript levels in transgenic plants led to a 58% to 76% decrease in \( \beta \)-ionone synthesis in the corollas of selected petunia lines, indicating a significant role for this enzyme in volatile synthesis. Quantitative reverse transcription-PCR analysis revealed that PhCCD1 is highly expressed in corollas and leaves, where it constitutes approximately 0.04% and 0.02% of total RNA, respectively. PhCCD1 is light-inducible and exhibits a circadian rhythm in both leaves and flowers. \( \beta \)-Ionone emission by flowers occurred principally during daylight hours, paralleling PhCCD1 expression in corollas. The results indicate that PhCCD1 activity and \( \beta \)-ionone emission are likely regulated at the level of transcript.

Polycomb group (PcG) proteins play an important role in developmental and epigenetic regulation of gene expression in fruit fly (Drosophila melanogaster) and mammals. Recent evidence has shown that Arabidopsis homologs of PcG proteins are also important for the regulation of plant development. The objective of this study was to characterize the PcG homologs in maize (Zea mays). The 11 cloned PcG proteins from fruit fly and the Enhancer of zeste \( [E(z)] \), extra sex combs (esc), and Enhancer of Polycomb \( [E(Pc)] \) homologs from Arabidopsis were used as queries to perform TBLASTN searches against the public maize expressed sequence tag database and the Pioneer Hi-Bred database. Maize homologs were found for \( E(z) \), esc, and \( E(Pc) \), but not for Polycomb, pleiohomeotic, Posterior sex combs, Polyclomblke, Additional sex combs, Sex combs on midleg, polyhometco, or multi sex combs. Transcripts of the three maize Enhancer of zeste-like genes, Mez1, Mez2, and Mez3, were detected in all tissues tested, and the Mez2 transcript is alternatively spliced in a tissue-dependent pattern. Zea mays fertilization independent endospermm1 (ZmFie1) expression was limited to developing embryos and endosperms, whereas ZmFie2 expression was found throughout plant development. The conservation of \( E(z) \) and esc homologs across kingdoms indicates that these genes likely play a conserved role in repressing gene expression.
organic species of Se (e.g. selenate, selenite, and Se-methionine [Met]) into gaseous Se forms (e.g. dimethylselenide), is a potentially important means of removing Se from contaminated environments. Before attempting to genetically enhance the efficiency of Se phytovolatilization, it is essential to elucidate the enzymatic pathway involved and to identify its rate-limiting steps. The present research tested the hypothesis that S-adenosyl-L-Met:L-Met S-methyltransferase (MMT) is the enzyme responsible for the methylation of Se-Met to Se-methyl Se-Met (SeMM). To this end, we identified and characterized an Arabidopsis T-DNA mutant knockout for MMT. The lack of MMT in the Arabidopsis T-DNA mutant plant resulted in an almost complete loss in its capacity for Se volatilization. Using chemical complementation with SeMM, the presumed enzymatic product of MMT, we restored the capacity of the MMT mutant to produce volatile Se.

Overexpressing MMT from Arabidopsis in Escherichia coli, which is not known to have MMT activity, produced up to 10 times more volatile Se than the untransformed strain when both were supplied with Se-Met. Thus, our results provide in vivo evidence that MMT is the key enzyme catalyzing the methylation of Se-Met to SeMM.


http://www.plantphysiol.org/cgi/content/abstract/pp.104.056010v1

In cotton (Gossypium hirsutum) the enzyme (+)-{delta}-cadinene synthase (CDNS) catalyzes the first committed step in the biosynthesis of cadinane-type sesquiterpenes, such as gossypol, that provide constitutive and inducible protection against pests and diseases. A cotton cDNA clone encoding CDNS (cdn1-C4) was isolated from developing embryos and functionally characterized. Southern analysis showed that CDNS genes belong to a large multigene family, of which five genomic clones were studied, including three pseudogenes and one gene that may represent another subfamily of CDNS. CDNS expression was shown to be induced in cotton infected with either the bacterial blight or verticillium wilt pathogens. Constructs for the constitutive or seed-specific antisense suppression of cdn1-C4 were introduced into cotton by Agrobacterium-mediated transformation. Gossypol levels were not reduced in the seeds of transformants with either construct, nor was the induction of CDNS expression affected in stems of the constitutive antisense plants infected with Verticillium dahliae Kleb. However, the induction of CDNS mRNA and protein in response to bacterial blight infection of cotyledons was completely blocked in the constitutive antisense plants. These results suggest that cdn1-C4 may be involved specifically in the bacterial blight response and that the CDNS multigene family comprises a complex set of genes differing in their temporal and spatial regulation and responsible for different branches of the cotton sesquiterpene pathway.


http://www.plantphysiol.org/cgi/content/abstract/134/3/979

Dihydroflavonol-4-reductase (DFR; EC1.1.1.219) catalyzes a key step late in the biosynthesis of anthocyanins, condensed tannins (proanthocyanidins), and other flavonoids important to plant survival and human nutrition. Two DFR cDNA clones (MtDFR1 and MtDFR2) were isolated from the model legume Medicago truncatula cv Jemalong. Both clones were functionally expressed in Escherichia coli, confirming that both encode active DFR proteins that readily reduce taxifolin (dihydroquercetin) to leucocyanidin. M. truncatula leaf anthocyanins were shown to be cyanidin-glucoside derivatives, and the seed coat proanthocyanidins are known catechin and epicatechin.
derivatives, all biosynthesized from leucocyanidin. Despite high amino acid similarity (79% identical), the recombinant DFR proteins exhibited differing pH and temperature profiles and differing relative substrate preferences. Although no pelargonidin derivatives were identified in M. truncatula, MtDFR1 readily reduced dihydrokaempferol, consistent with the presence of an asparagine residue at a location known to determine substrate specificity in other DFRs, whereas MtDFR2 contained an aspartate residue at the same site and was only marginally active on dihydrokaempferol. Both recombinant DFR proteins very efficiently reduced 5-deoxydihydroflavonol substrates fustin and dihydrorobinetin, substances not previously reported as constituents of M. truncatula. Transcript accumulation for both genes was highest in young seeds and flowers, consistent with accumulation of condensed tannins and leucoanthocyanidins in these tissues. MtDFR1 transcript levels in developing leaves closely paralleled leaf anthocyanin accumulation. Overexpression of MtDFR1 in transgenic tobacco (Nicotiana tabacum) resulted in visible increases in anthocyanin accumulation in flowers, whereas MtDFR2 did not. The data reveal unexpected properties and differences in two DFR proteins from a single species.

Plant Physiology and Biochemistry (3)


http://www.sciencedirect.com/science/article/B6VRD-494P6F5-3/2/d45a1dd5d210dbe7754cc8e3a6ac42ee

Aspartate transcarbamoylase (ATCase; EC 2.1.3.2) catalyzes the committed step in the de novo synthesis of pyrimidine nucleotides. We investigated the effects of N-(phosphonacetyl)-L-aspartate (PALA), a transition-state analog inhibitor of ATCase, on seedling growth and development, RNA and soluble protein contents, ATCase activity and enzyme protein levels, and pyrB gene expression in Arabidopsis thaliana L. cv. "Columbia". In vitro, PALA was a potent inhibitor of ATCase, with an apparent Ki = 22 nM. After 5 d of treatment with 1 mM PALA, seedlings exhibited delayed germination, inhibition of cotyledon expansion, leaf development and root growth, and general chlorosis. Total RNA contents of these seedlings were decreased by 81% and total soluble protein contents decreased by 74%, compared with untreated control plants. Levels of pyrB mRNA increased about tenfold in PALA-treated plants, while ATCase activity and enzyme protein levels increased twofold. Plants grown on media containing a lower (0.1 mM) concentration of PALA did not exhibit significant inhibition of growth until after 9 d of treatment, but had markedly reduced RNA contents (40% of controls) and elevated pyrB mRNA levels (fourfold increase) after 12 d of treatment.


Arabidopsis seedlings grown for 14 d without phosphate (P) exhibited stunted growth and other visible symptoms associated with P deficiency. RNA contents in shoots decreased nearly 90%,
relative to controls. In shoots, expression of Pht1;2, encoding an inducible high-affinity phosphate transporter, increased threefold, compared with controls, and served as a molecular marker for P limitation. Transcript levels for five enzymes (aspartate transcarbamoylase, ATCase, EC 2.1.3.2; carbamoyl phosphate synthetase, CPSase, EC 6.3.5.5; UMP synthase, EC 2.4.1.10, EC 4.1.1.23; uracil phosphoribosyltransferase, UPRTase, EC 2.4.2.9; UMP kinase, EC 2.7.1.14) increased 2-10-fold in response to P starvation in shoots. These enzymes, which utilize phosphorylated intermediates at putative regulated steps in de novo synthesis and salvaging pathways leading to UMP and pyrimidine nucleotide formation, appear to be coordinately regulated, at the level of gene expression. This response may facilitate pyrimidine nucleotide synthesis under P limitation in this plant. Expression of P-dependent and P-independent phosphoribosyl pyrophosphate (PRPP) synthases (PRS2 and PRS3, respectively) which provide PRPP, the phosphoribosyl donor in UMP synthesis via both de novo and salvaging pathways, was differentially regulated in response to P limitation. PRS2 mRNA levels increased twofold in roots and shoots of P-starved plants, while PRS3 was constitutively-expressed. PRS3 may play a novel role in providing PRPP to cellular metabolism under low P availability.


http://www.sciencedirect.com/science/article/B6VRD-45SGTMS-9/2/bfc247c5f4c786e02dd88e66577a416d

Little is known about gene expression during fruit ripening of apricot (Prunus armeniaca L. cv. Bergeron), especially for enzymes involved in cell wall modifications. A partial cDNA clone encoding a protein homologous to expansin was isolated from a ripe apricot fruit cDNA library. This clone was used to isolate two full-length expansin cDNAs, Pa-Exp1 (accession no. U93167) and Pa-Exp2 (accession no. AF038815) from the same cDNA library. The predicted polypeptides encoded by these two cDNAs are different and belong to the [alpha]-expansin family; Pa-Exp1 and Pa-Exp2 are two different members of a multigene family. These two clones are mostly expressed in fruit, during its ripening. Pa-Exp1 mRNA accumulated abundantly at the half-ripe stage of fruit development and decreased thereafter. Pa-Exp2 mRNA level increased from the immature-green stage to the half-ripe stage where it peaked before declining. During the ripening process, Pa-Exp1 and Pa-Exp2 gene expression appeared to be positively correlated with fruit size. Post-harvest treatments by air, ethylene, and 1-methyl cyclopropene led us to conclude that Pa-Exp1 appears to be developmentally down-regulated by ethylene while Pa-Exp2 is not affected. The relationship between Pa-Exp1, Pa-Exp2 and the softening process is also discussed.

Plant Science (34)


http://www.sciencedirect.com/science/article/B6TBH-3YVDPXV-8/2/fb1276ab533b29cbe55de6153f127eed
Transgenic tobacco plants producing the synthetic antimicrobial peptide D4E1, encoded by a gene under the control of an enhanced cauliflower mosaic virus 35S RNA promoter, were obtained by Agrobacterium-mediated transformation. Successful transformation was demonstrated by PCR and Southern hybridization analysis of tobacco DNAs. Expression of the synthetic D4E1 gene was shown by RT-PCR of tobacco mRNA. Crude protein extracts from leaf tissue of transformed plants significantly reduced the number of fungal colonies arising from germinating conidia of Aspergillus flavus and Verticillium dahliae by up to 75 and 99%, respectively, compared to extracts from plants transformed with pBl121. Compared to negative controls, tobacco plants expressing the D4E1 gene showed greater levels of disease resistance in planta to the fungal pathogen, Colletotrichum destructivum, which causes anthracnose.


http://www.sciencedirect.com/science/article/B6TBH-42Y11N6-4/2/7b54ff298e2d75249705568a0eba2e4e

Analysis of a sugarcane (Saccharum spp.) EST (expressed sequence tag) library of 8678 sequences revealed approximately 250 microsatellite or simple sequence repeats (SSRs) sequences. A diversity of dinucleotide and trinucleotide SSR repeat motifs were present although most were of the (CGG)n trinucleotide motif. Primer sets were designed for 35 sequences and tested on five sugarcane genotypes. Twenty-one primer pairs produced a PCR product and 17 pairs were polymorphic. Primer pairs that produced polymorphisms were mainly located in the coding sequence with only a single pair located within the 5’ untranslated region. No primer pairs producing a polymorphic product were found in the 3’ untranslated region. The level of polymorphism (PIC value) in cultivars detected by these SSRs was low in sugarcane (0.23). However, a subset of these markers showed a significantly higher level of polymorphism when applied to progenitor and related genera (Erianthus sp. and Sorghum sp.). By contrast, SSRs isolated from sugarcane genomic libraries amplify more readily, show high levels of polymorphism within sugarcane with a higher PIC value (0.72) but do not transfer to related species or genera well.


http://www.sciencedirect.com/science/article/B6TBH-47T1J5B-22/2/a5a2f1479ed52be9b36883b2bcf158ce

An RFLP analysis of five date palm (Phoenix dactylifera L.) elite cultivars (cvs. Barhee, Deglet Nour, Khalassa, Khadrawy, and Medjool) has been performed on shoot leaves surrounding the shootips used to initiate tissue culture. Total DNA digested by EcoRI was hybridized with cDNA probes randomly selected from a cDNA library constructed from highly organogenic calli of cv. Boustammi Noire, and with a heterologous 1.7-kb nuclear rDNA fragment, amplified during a polymerase chain reaction (PCR) of jojoba genomic DNA. Discrimination among the five cultivars was easily made with cDNA probe 1, which was highly polymorphic. A polymorphismm among cultivars was also observed by amplification with random primers of total DNA extracted from shoot leaves. Preliminary attempts made to asses the extent of variability at the DNA level as a result of tissue culture, are also reported. With the availability of probes such as cDNA 1, the use of RFLP for rapid and reliable cultivar identification, and screening of cultivated populations with economically important traits in date palm growing countries is now conceivable.
Cinnamyl alcohol dehydrogenase (CAD) (EC 1.1.1.195) catalyses the final step in lignin precursor synthesis reducing the cinnamyl aldehydes (para-coumaryl, coniferyl and sinapyl aldehydes) to the corresponding alcohols in the presence of NADPH. In this paper, we report the molecular cloning and characterisation of a Eucalyptus globulus genomic fragment encoding CAD2, and the corresponding full-length cDNA isolated from young stem material. This was achieved using the polymerase chain reaction-based method known as rapid amplification of cDNA ends, with oligonucleotide primers corresponding to regions of homology between CAD-encoding sequences from other eucalypt species. The identity of the clones was inferred by sequence data comparison and the cDNA sequence (1423 bp) was found to encode a protein of 356 amino acid residues. The CAD2 transcript was most abundant in stem, followed by root and midrib tissues, which corresponds with the role of lignin in water retention in plants and in providing mechanical support. Low level expression was also observed in leaf tissue. Southern blot analysis revealed a single CAD gene in this species with the presence of possibly different allelic representations.

The role of ethylene in aroma biosynthesis of apple fruits was investigated using transgenic 'Greensleeves' apple trees suppressed for ACC-oxidase or ACC-synthase enzyme activity, and an ethylene action inhibitor (1-methycyclopropene, 1-MCP). In the transgenic lines and 1-MCP treated fruit, reductions higher than 90% in ethylene biosynthesis and respiration rates were observed in apples held at 20 [deg]C for 14 days. We observed a major reduction in ester production in the ethylene-suppressed lines and in the 1-MCP treated fruit, with only slight differences in the levels of alcohol and aldehyde volatiles under these conditions. The activity of alcohol acyl-CoA transferase (AAT), a key enzyme in ester biosynthesis, showed an ethylene dependent pattern of regulation. Additionally, gene expression levels of specifically an AAT clone were highly regulated by ethylene. In contrast, activity and expression levels of alcohol dehydrogenase (ADH) were not affected by changes in the levels of endogenous ethylene. These results suggest that ethylene is involved in ester biosynthesis in apple via regulation of AAT.

A cDNA for spermidine synthase (SPDS), which converts putrescine to spermidine using decarboxylated S-adenosylmethionine as a co-factor, has been isolated from Coffea arabica. When the SPDS cDNA is expressed in an SPDS-deficient E. coli mutant, the recombinant protein shows high SPDS activity. The C. arabica SPDS possesses the co-factor binding motifs which
have been proposed for S-adenosylmethionine, and its amino acid sequence is similar to other plant SPDSs. The SPDS transcripts have been observed in roots, green stems, old and young leaves, and accumulated to a higher level in rapid growing tissues, such as green stems and younger leaves, compared to old leaves. In callus tissues, it has been expressed in all stages, even though callus growth is very slow during somatic embryogenesis.


We have developed an efficient direct DNA transfer procedure for the facile engineering of Catharanthus roseus cell cultures. Particle bombardment of callus derived from leaf material permitted rapid selection and establishment of transgenic cell lines. Transgenic callus were recovered at a frequency of between 60-80% of total callus bombarded with a single plasmid. Bombardment using two separate plasmids resulted in a 25-60% frequency of transgenic callus recovered, up to 90% containing both input plasmids. Between 10-20 g FW of transgenic material was produced within 3 months of bombardment, providing sufficient material for molecular and biochemical analyses. We developed two complementary systems allowing selection on either hygromycin or kanamycin to permit re-transformation using plasmids carrying additional genes of interest. Use of leaf tissue as explant for transformation avoids time-consuming and labor intensive procedures involving suspension cultures. We provide molecular data on integration and expression of selected and non selected transgenes in a number of transgenic callus lines. Transgene integration events for co-transformed plasmids were relatively simple, occurring at one or two sites in the genome for most of the lines we analysed. Molecular analysis of callus resulting from co-transformation experiments using two different plasmids revealed that in nine of 10 putative transgenic lines we selected for analysis both plasmids had integrated into the genome. RNA gel-blot analysis and histochemical staining showed that an unselected transgene, gusA, was expressed in seven of the ten lines we analysed.


http://www.sciencedirect.com/science/article/B6TBH-482YHVX-3/2/0c32d03d3782777c95b71b22f21a554fe

The effect of high temperature on starch accumulation, starch granule populations, and expression of genes encoding key enzymes for starch biosynthesis was examined during grain development in wheat (Triticum aestivum L. cv. Butte 86). High temperature applied from anthesis to maturity reduced the duration of starch accumulation. Starch accumulation ceased approximately 6 days earlier for grain produced under a 37/17 [deg]C (day/night) regimen and 21 days earlier under a 37/28 [deg]C (day/night) regimen than for grain produced under a 24/17 [deg]C (day/night) regimen. Compared to the 24/17 [deg]C regimen, starch content was approximately 19% less for mature grain produced under the 37/17 [deg]C regimen and 58% less under the 37/28 [deg]C regimen. Based on relative volume, the smaller type B starch granules were the predominant class in mature grain produced under the 24/17 and 37/17 [deg]C regimens, whereas the larger type A granules were predominant in grain produced under the 37/28 [deg]C regimen. Under the 24/17 [deg]C regimen, steady state transcript levels for ADP-glucose pyrophosphorylase, starch synthases I, II, and III, granule-bound starch synthase, and starch branching enzymes I and II were highest from 12-16 days post-anthesis (dpa). Under the 37/17 [deg]C regimen, steady state levels of these transcripts followed the same temporal
pattern, but were substantially lower. Under the 37/28 [deg]C regimen, transcript levels peaked earlier, at 7 dpa. The high temperature regimes reduced the relative levels of transcripts for starch synthase more than the other starch biosynthetic enzymes.


We isolated a cDNA clone (ATMRK1) with sequence similarity to mammal mixed-lineage kinase homologues and Raf protein kinase homologues in the catalytic domain from Arabidopsis thaliana using the polymerase chain reaction (PCR). The ATMRK1 cDNA encodes a 391-amino acid polypeptide containing all 11 conserved regions of the catalytic domains of protein kinases. The catalytic domain of the putative ATMRK1 protein has sequence similarity with those of protein kinases that belong to mammalian mixed-lineage kinases, MLK-2 (33%) and c-Raf-1 protein kinase (26%). The ATMRK1 protein has the highest homology with plant protein kinases, Glycine max PK6 (36%), Arabidopsis protein kinases ATN1 (36%) and CTR1 (34%). CTR1 encodes Raf family protein kinase and negatively regulates ethylene signal transduction. The phylogenetic tree shows that the plant protein kinases ATMRK1, GmPK6 and ATN1 are classified into the same cluster close to mixed-lineage and Raf kinases. DNA gel blot analysis and a search of the plant expressed sequence tag (EST) database showed the existence of several ATMRK1-related genes in the Arabidopsis genome. RNA gel blot analysis revealed that transcripts of ATMRK1 are detected in all tissues. The highest level of its expression was obtained from root tissues.


The main objective of this study was to assess the extent of genetic diversity detected by RAPD (random amplified polymorphic DNA) technique among 15 varieties of common bread wheat (Triticum aestivum L.). The slow development of genetic linkage maps of wheat using conventional molecular marker strategies is attributed to the limited number of RFLPs (restriction fragment length polymorphisms) between wheat genotypes. Recently, RAPDs have been observed between closely related genotypes in several other species. We have used a set of 40 single arbitrary primers (10-mers) for the PCR (polymerase chain reaction) -mediated amplification of random genomic DNA fragments from wheats. Eighty percent of the primers yielded distinct electrophoretic profiles which could be scored. Out of 109 amplified fragments, 71 (65%) were polymorphic in these wheat cultivars. These results have assisted in the development of a dendrogram suggesting genetic relationships among these genotypes. Moreover, most of the spring and winter wheats were clustered together in this dendrogram based on Jaccard’s coefficients. These results will be useful in the identification of suitable parents for the development of a mapping population for tagging agronomically important traits in wheat.

We have isolated two closely related genes, Sbe2.1 and Sbe2.2, encoding isoform II of starch branching enzyme (SBE) from an Arabidopsis thaliana genomic library. Although a partial cDNA clone encoding the isoform I of the potato SBE was used as probe, no clones corresponding to this isoform were found in the Arabidopsis library. Sbe2.1 was completely and Sbe2.2 partially sequenced. PCR-screening of a large number of individual plants using gene specific primers, revealed that both genes were independently present in the genome of A. thaliana. The Sbe2.1 gene consisted of 18 exons interrupted by 17 introns and the open reading frame comprised 2574 bp encoding a protein with a calculated molecular mass of 97660 Da. The promoter region of both Sbe2 genes contained potential sucrose responsive elements (SURE1 and SURE2). In addition, a potential light responsive element (G-box) was present in the Sbe2.1 promoter. Northern blot analysis using gene specific probes, showed a differential accumulation of transcripts from the Sbe2 genes as a response to light. While the level of Sbe2.1 transcripts increased and decreased during light and dark, respectively, those transcribed from the Sbe2.2 gene did not change significantly. However, transcripts from both Sbe2 genes accumulated strongly when plants were incubated in light and with exogenous supply of either glucose, fructose or sucrose. No significant changes in Sbe2 transcript levels were detected in plants incubated with sorbitol under conditions of dark or light, indicating that the expression of Sbe2 genes is stimulated by specific carbohydrate signals.


The expression of the UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene has been shown to be critical for anthocyanin biosynthesis in the grape berry. Using white cultivars and bud sports with red skin, we examined the expression of seven anthocyanin biosynthetic genes including the UFGT gene and compared the coding/promoter sequences of the UFGT gene. Northern blot analysis showed that the seven anthocyanin biosynthetic genes were expressed coordinately at higher levels in the red-skin sports than in the white-skin progenitors of the sports. It was especially notable that UFGT gene expression was detected only in the red-skin sports and Kyoho. However, there were no differences in either coding or promoter sequences between Italia (Vitis vinifera) and its red-skin sport Ruby Okuyama, or between Muscat of Alexandria (V. vinifera) and the red-skin sport Flame Muscat. From these findings, the phenotypic change from white to red in the sports is thought to be the result of a mutation in a regulatory gene controlling the expression of UFGT.


Carnation petals exhibit autocatalytic ethylene production and wilting during senescence. The autocatalytic ethylene production is caused by the expression of 1-aminocyclopropane-1-
carboxylate (ACC) synthase and ACC oxidase genes, whereas the wilting of petals is related to the expression of the cysteine proteinase (CPase) gene. So far, it has been believed that the ethylene production and wilting are regulated in concert in senescing carnation petals, since the two events occurred closely in parallel with time. In the present study, we investigated the expression of these genes in petals of a transgenic carnation harboring a sense ACC oxidase transgene and in petals of carnation flowers treated with 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide (DPSS). In petals of the transgenic carnation flowers, treatment with exogenous ethylene caused accumulation of the transcript for CPase and in-rolling (wilting), whereas it caused no or little accumulation of the transcripts for ACC oxidase and ACC synthase and negligible ethylene production. In petals of the flowers treated with DPSS, the transcripts for ACC synthase and ACC oxidase were accumulated, but no significant change in the level of the transcript for CPase was observed. These results suggest that the expression of ACC synthase and ACC oxidase genes, which leads to ethylene production, is differentially regulated from the expression of CPase, which leads to wilting, in carnation petals.

Kozik, A., R. Heidstra, et al. (1995). "Pea lines carrying sym1 or sym2 can be nodulated by Rhizobium strains containing nodX; sym1 and sym2 are allelic." Plant Science 108(1): 41.

http://www.sciencedirect.com/science/article/B6TBH-3XVH3PR-1K/2/59e3a545611efca527aeey57179717f3

In wild pea varieties two genes, sym1 and sym2, have been identified that cause resistance to European Rhizobium leguminosarum bv. viciae (Rlv) strains. The sym2 gene has previously been studied in some detail and it was shown that the additional nodulation gene nodX is sufficient to overcome the sym2 controlled nodulation resistance. Here we characterize the sym1 gene. We show that the resistance conferred by sym1 can be overcome by the introduction of nodX in European Rlv strains, indicating that sym1 just as sym2 is involved in Nod factor recognition. Both sym1 and sym2 display a recessive or dominant nature depending on the Rlv strain used for inoculation. Furthermore, introgression lines containing either sym1 or sym2 are able to form nodules with Rlv strain 248 at 26[deg]C, but not at 18[deg]C, indicating that both sym1 and sym2 have a temperature sensitive nature. sym2 was mapped on the pea RFLP map. We found that sym1 maps in the same region of chromosome 1 as sym2. By crossing sym1 and sym2 containing introgression lines we demonstrate that sym1 and sym2 are allelic.


http://www.sciencedirect.com/science/article/B6TBH-486BPW1-1/2/9586493384abadd08376051557f1e280

We conducted differential screening to obtain cDNAs showing that gene expression is highly associated with the transformation from immature pollen to embryogenic cell, so-called embryogenic dedifferentiation of pollen, in a Nicotiana tabacum pollen culture system and analyzed their expression and sequences. Seventy-seven cDNA clones were independently isolated and distinguished into 16 groups based on their sequences. The groups were further categorized into two classes, Class I and II, based on the gene expression pattern of the representative clone of each group under various pollen culture conditions arranged for examining the coincidence with the dedifferentiation. The 13 groups in Class I showed prominent expression under the conditions allowing or facilitating pollen dedifferentiation and the expression level increased earlier than A-type cyclin genes, but they were not markedly expressed in the cell populations rich in S-phase cells, i.e. young anthers with pollen mother cells, BY-2 cells at the growth phase and early phase embryos derived from immature pollen. The other three groups in
Class II encoded homologs to H1 histone, H2A histone and minichromosome maintenance (MCM) protein, respectively. The level of their transcripts increased during dedifferentiation, but it was also high in anthers containing pollen mother cells and in the proliferating BY-2 cells indicating that their expression is coincident with the S phase but not with dedifferentiation. These findings suggest that pollen dedifferentiation is a complex process accompanied with the reentrance of cell cycle and unknown events probably caused by specific expression of many genes, at least, listed in Class I. These genes should be used as reliable markers and important clues for further studies on the molecular mechanism of dedifferentiation.


http://www.sciencedirect.com/science/article/B6TBH-46P1KGF-4/2/f7d0b3aeeec79e0c10a2b75f7bbe0d41c

Several phosphoproteins, Nicotiana tabacum L. embryogenic pollen-abundant phosphoproteins (NtEPs), characteristically appear in the dedifferentiation process from immature pollen grains to embryogenic cells in a pollen culture system. Among NtEPs we focused our attention on three proteins (NtEPb1-b3) which showed the highest correlation with the dedifferentiation and possessed different pi values and similar molecular weights (ca. 22 kDa). Using probes designed from the N-terminal amino acid sequence common to the three, we isolated 14 clones of cDNA belonging to three similar sequences which probably correspond to NtEPb1-b3. The predicted amino acid sequences showed moderate homology to NtEPc, several type-1 copper-binding glycoproteins and a kind of early nodulin. The level of the transcripts for NtEPbs is highly associated with the pollen dedifferentiation but not with pollen maturation nor with cell division accompanied by meiosis or proliferation of BY-2 cells. Such an expression manner was distinguished from that of a gene coding for A-type cyclin (Ntcyc 25), indicating that NtEPb genes are not under cell cycle control. These results suggest that there exist genes related to an unknown event other than the reentrance of cell cycle in the dedifferentiation process of immature pollen that may be important for acquisition of embryogenic competence.


http://www.sciencedirect.com/science/article/B6TBH-4B0NVHH-5/2/68f2c3f8d40f09f4ed69dcf70a6b5f70

Cinnamoyl-CoA reductase (CCR; EC 1.2.1.44) catalyzes the reduction of cinnamoyl acid-CoA esters into their corresponding aldehydes, the first step in the biosynthesis of lignin. The synthesis of lignin monomers is strictly associated with the activity of this enzyme which occurs in significant concentrations in lignin producing tissues. A 1207 bp cDNA (LpCCR) encoding a polypeptide of 344 amino acids with a predicted molecular mass of 37.4 kDa was isolated from a ryegrass (Lolium perenne) stem cDNA library. The identity of the LpCCR was established by comparison of the deduced polypeptide sequence with other isolated plant CCR enzymes. A motif, NWYCY, representing a putative CCR active site is conserved in the encoded LpCCR amino acid sequence. The encoded polypeptide exhibits sequence similarity to CCRs from different plants, the highest identities (87 and 86%, respectively) being to CCRs from Festuca arundinacea and Hordeum vulgare. Phylogenetic analysis shows that LpCCR and CCR from other monocotyledons form a group distinct from dicot CCRs. Genomic Southern blot hybridization demonstrated that LpCCR probably is represented as a single-copy gene in the ryegrass genome. The isolation of a single genomic CCR clone (gLpCCR) from a ryegrass leaf library further supports this observation. The gLpCCR contains four introns, the same number found in
the Eucalyptus gunnii gCCR. Computer analysis of a 1333 bp 5'-flanking region of gLpCCR suggests the presence of binding sites for a MYB transcription factor and a binding site for a P-box factor of the DoF class transcription factors. In agreement with the involvement of CCR in lignification, the ryegrass CCR mRNA was detected in stem tissue only. The isolation and characterization of gLpCCR provides tools for genetically modifying plants with reduced lignin content thereby increasing forage digestibility.


http://www.sciencedirect.com/science/article/B6TBH-47STS3K-YW/2/a2fdacb78ca6184b4849c5b9c05e27f7

A P4-chitinase genomic sequence was isolated from a bean (Phaseolus vulgaris) genomic library using a P4-ch cDNA. The complete sequence of the P4-ch gene was determined. Primer extension analysis allowed the identification of the transcriptional start site located 79 bp upstream of the translational initiation codon. The gene is interrupted by an intervening sequence. In the 5' upstream region, a TATT box occurs in place of a TATA at position -33, while a typical CAAT box is found at position -33 with respect to the transcription initiation site. Gibberellic acid, heat shock and salicylic acid regulatory responsive sequences were also identified. Transient expression of chimeric genes in tobacco protoplasts indicated that all the elements required for expression of the coding sequences are present within the first 600 bp of P4-ch 5' flanking DNA. Various stress conditions such as wounding, salicylic acid and NaCl treatments, heat and cold stress have been applied to the plants. Whereas wounding, NaCl treatment and cold stress are ineffective, transcription of P4-mRNAs is induced upon salicylic acid treatment and, surprisingly, in response to heat stress. P4-chitinase is induced during germination and seems to be constitutively expressed in roots of mature plants.


http://www.sciencedirect.com/science/article/B6TBH-4DW3JY0-1/2/ad430c2e39557a93de1ddbb155e370cc

2,4,6-trinitrotoluene (TNT) is a nitro-substituted xenobiotic explosive that is toxic to plants and animals. Plants absorb and metabolize TNT, but the pathways are uncertain and plant responses at the molecular level have not been adequately characterized. We analyzed gene expression in response to relatively long-term exposure to TNT at low and high concentration in Arabidopsis through the use of cDNA microarrays. Arabidopsis seedlings were grown on media containing 1 and 10 [μM] TNT, concentrations that were empirically determined by plant growth analysis. Microarray analysis revealed that a total of 52 genes were significantly upregulated, and 47 genes were downregulated in response to TNT at a 1.7-fold cut-off for differential gene expression. A substantial number of these genes have predicted functions in cell defense and detoxification. Conserved motifs were discovered in the promoter region of co-regulated genes, some of which are potentially novel cis-regulatory elements. With follow-up real time RT-PCR, we confirmed findings from the microarray experiments and examined the regulation of selected genes to two other xenobiotic substances: the explosive RDX and thioglycol. Results showed that increased transcription of At5g61600 encoding for a DNA-binding protein in shoots is specific to TNT and increased transcription of At5g42530 encoding for an unknown protein in shoots is specific to both TNT and RDX.
We have cloned a cDNA encoding a putative methionine synthase from barley leaves by differential display. The full-length cDNA contained an open reading frame (2295 bp) encoding a deduced 765 amino acid polypeptide without any typical signal sequence, suggesting that it is localized in the cytosol. Expression of this gene was induced by salt stress in barley leaves. The gene expression was induced by light, but more strongly induced under salt, drought and cold stresses and by treatment with ABA or H2O2. A complementation test using a yeast mutant lacking the ability to synthesize methionine showed that this gene complements the yeast mutant under both non-stress and high-salinity (1 M NaCl) conditions. Although plant methionine synthase is known to be induced at the level of mRNA but not at that of protein, we found that the protein level of methionine synthase is also significantly increased in barley leaves under salt stress.

The exposure of Chlamydomonas reinhardtii to environmental stress, such as that caused by the explosive 2,4,6-trinitrotoluene (TNT) can alter its gene expression. Expression analysis was conducted using a microarray composed of 3079 Chlamydomonas ESTs to characterize the broad range of responses of gene expression exposed to this common ordnance compound. TNT treatment conditions were determined by growth analysis of Chlamydomonas in 0-5 [μg/mL] TNT. One and 3 [μg/mL] were used for microarray analysis since 1 [μg/mL] of TNT did not decrease the cell count after 7 days of treatment, whereas 3 [μg/mL] of TNT was the maximum TNT concentration that allowed growth, respectively. Transcriptional profiling revealed that approximately 158 responsive genes were differentially expressed representing several functional categories. Genes responsible for photosynthesis, energy metabolism and oxidative stress were upregulated in the presence of TNT, while the expression of cell wall related genes were downregulated. Several unidentified genes were also affected. The microarray results were validated using real-time RT-PCR for a subset of genes. Information from the microarray analysis can be used to engineer algae-based sensors to signal TNT exposure in addition to potential explosives cleanup applications.
medium containing 25 mg/l of kanamycin, the inoculated leaf explants formed meristematic centers with buds and embryo-like structures that successively developed into putative transformed shoots, when transferred onto medium without growth regulators. Under suitable conditions, three days of cocultivation on medium containing BAP and NAA, the highest transformation frequency was 5.4%. Histochemical staining for the [beta]-glucuronidase (GUS) activity provided evidence for transformation in different tissues and organs of transgenic plants. Integration of foreign DNA into genomic H. annuus x H. tuberosus DNA was demonstrated by Southern analysis.


http://www.sciencedirect.com/science/article/B6TBH-3T3JCKY-2/2/79c7634a8b1e32511839801123c233db

We examined the effect of nitrate on the expression of the NADPH producing enzymes of the oxidative pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in maize seedlings (Zea mays L. W64A x A182E). In extracts of 5 day old maize roots and leaves treated with 10 mM KNO3, G6PDH and 6PGDH activities increased by 44 and 53%, respectively, relative to untreated roots. In isolated plastids from KNO3 treated roots, G6PDH and 6PGDH specific activities were more than 25- and 12-fold higher than in the untreated control. Western blot analysis showed higher levels of 6PGDH protein in root plastid extracts from KNO3 treated plants. The data suggest that KNO3 specifically affects the plastidic forms of G6PDH and 6PGDH. Three classes of 6PGDH cDNA were identified in maize roots. Of these, one cDNA hybridized with a transcript that accumulated rapidly and transiently in response to low concentrations of external nitrate. The accumulation of this transcript was not affected by pretreating plants with 50 [mu]g/ml cycloheximide, which was previously shown to inhibit cytoplasmic protein synthesis in maize roots by more than 85% (Gowri et al., Plant Mol. Biol. 26 (1998) 679). Neither NH4+ nor K+ treatment affected transcript accumulation. The data indicate coordinated regulation of genes and enzymes required for NO3-assimilation and NADPH production in root plastids.


http://www.sciencedirect.com/science/article/B6TBH-3WNMGG3-7/2/4568b3f4b4ec1646a3844bcb23943cc0

PCR amplification from leaf tissue has become an integral part of many plant molecular biology applications including screening for transformants, plant breeding, and molecular ecology. We have adapted an existing method to produce a simple protocol for the amplification of single or multicopy genes directly from leaf discs, particularly useful when screening large numbers of individuals.


http://www.sciencedirect.com/science/article/B6TBH-4B5J8VT-
ALS gene from susceptible and field-selected Papaver rhoeas populations resistant to ALS-inhibitor herbicides was studied. The full-length cDNA and genomic sequence coding for acetolactate synthase (ALS) of a susceptible population of poppy was cloned and sequenced. Some peculiarities in poppy ALS gene were identified aligning the sequences in GenBank. Southern analysis using a 800 bp fragment of ALS showed that poppy possesses a single copy of the gene. Partial ALS genomic DNAs from nine poppy populations resistant to ALS-inhibitor herbicides and four susceptible populations, collected in central and southern Italy where resistance to ALS-inhibitors is increasing due to repeated use of sulfonylureas, were amplified and sequenced. Comparison of the coding sequences identified three independent point mutations leading to different amino acid substitutions in the deduced polypeptide sequence. The three point mutations, all at proline 197 (CCT) (based on Arabidopsis numbering) in the conserved domain A of the gene, included a change of Pro to His (CAT), to Thr (ACT) or to Ser (TCT). These mutations cause similar cross-resistance patterns. Analysis of the progeny of two crosses between resistant (R) and susceptible (S) biotypes indicated that resistance is inherited as a dominant monogenic trait, although seed dormancy may interfere with a correct segregation of R and S biotypes in the progeny. These are the first mutations completely characterised in poppy ALS gene that confer resistance to ALS-inhibitor herbicides.


http://www.sciencedirect.com/science/article/B6TBH-42RDSMC-5/2/29807e91989e4045bed6d25da78d1b04

A pathogenesis-related (PR) protein was purified from the seeds of Benincasa hispida, which is a medicinal plant and a member of the Cucurbitaceae family. Purification was achieved by using a procedure consisting of an acid treatment step followed by two chromatography steps. The protein is a basic protein with molecular mass of ~28 kDa. The sequences of the N-terminal 30 amino acids and four peptides generated from protease digestion were determined. These sequences indicated that the protein is an osmotin-like protein (OLP). Osmotin and OLPs are members of the thaumatin-like, PR-5 family of the PR proteins. A genomic clone of the gene encoding the protein was isolated and sequenced. The predicted protein has a signal peptide of 18 amino acids, and the mature protein has a molecular mass of 24.8 kDa with an isoelectric point of 7.67. The protein has 17 cysteine residues, of which 16 appear in the same positions as those appear in the sweet-tasting protein thaumatin and several other thaumatin-like proteins. Southern hybridization analysis indicated that the gene encoding the protein is a single copy gene. A computer-generated, three-dimensional model of the protein is presented.


http://www.sciencedirect.com/science/article/B6TBH-40SFG52-2/2/79c88f4e8cab3cc994806ef42be88677

Permatins are antifungal thaumatin-like proteins (TLPs) of the PR-5 family of pathogenesis-related proteins. They occur in many cereals, but little is known of their expression and roles. Permatin cDNA clones were produced and used to study expression in developing barley and oat seeds. Actin and CDC48 mRNAs declined rapidly following inoculation of barley spikes with
Fusarium graminearum. Despite this, permatin mRNA levels remained constant or increased slightly. Studies of permatin gene expression in healthy plants revealed that developing barley and oat seeds accumulate permatin mRNA in an unusual bimodal pattern. Permatin mRNA and protein are highly abundant around the time of pollination and then decrease rapidly to near-zero. A second peak occurs in the doughy stage of development. Antibody and DNA probe hybridization studies showed that expression initially occurs in the ovary wall and then switches to the aleurone and ventral furrow of developing seeds, reaching a peak in the doughy stage. Small amounts of permatin mRNAs also occur in certain vegetative tissues. The barley and oat permatin sequences provided sufficient comparisons between cereal TLPs to suggest that deletions or additions in specific elements could have led to the divergence of leaf- and seed-specific TLPs.


http://www.sciencedirect.com/science/article/B6TBH-48YVMWT-1/2/6d959a1ac460304850eea1f25d1e3ace

A chimeric gene using [beta]-glucuronidase (GUS) as a reporter gene under the control of a 1.3 kb 5'-flanking region of pck1 (involved in C4 photosynthesis in Urochloa panicoides) was introduced into rice and maize. GUS activity was detected in leaf blades, leaf sheaths and roots of transgenic rice plants and was detected at high levels in leaf blades and at low levels in leaf sheaths and roots of transgenic maize plants. The pck1 promoter drove the expression of GUS activity in transgenic maize following 6 h of illumination. In contrast, GUS activity was not induced in transgenic rice even after 24 h illumination. Histochemical analysis revealed that GUS staining was localized to bundle sheath cells and vascular bundles of both rice and maize transformants and GUS activity in bundle sheath cells of transgenic maize was induced by light. These results suggest that the 1.3 kb pck1 promoter contains cis-acting elements for preferential and abundant expression in bundle sheath cells of the leaf blade with light dependence in maize but rice lacks some trans-acting elements required for the expression controlled by pck1.


http://www.sciencedirect.com/science/article/B6TBH-3WYHT1T-5/2/d1a563f4bd1fcae7377101283b226c47

Chymopapain is one of the four known cysteine proteinases found in the latex of Carica papaya. DNA sequencing of clones derived from a leaf cDNA library identified five cDNA types coding for precursor chymopapains. All of these isoforms have a free cysteine residue at position 117, characteristic of chymopapain. Two of the isoforms possess a further free cysteine residue, which is not likely to be involved in disulphide bonds or the active site apparatus. Another amino acid substitution found in two of the isoforms at position 133 is predicted to lie in the S2 subsite of the substrate binding cleft. One of the prochymopapain isoforms was expressed in Escherichia coli. Protein was expressed as insoluble inclusion body material. This protein was solubilised, refolded and autocatalytically cleaved to yield mature chymopapain that had comparable kinetic constants to authentic native enzyme.

A cDNA clone, ATN1, coding for a novel protein kinase, was isolated from an Arabidopsis thaliana inflorescence cDNA library. The deduced ATN1 protein sequence of 356 amino acid residues contains all the invariant hallmarks of the protein kinase superfamily. The ATN1 protein is however unusual in that it contains not only amino acid motifs characteristic of the protein serine/threonine kinase family, but also residues typical of protein tyrosine kinases. The ATN1 protein is not closely related to any other protein kinases characterised to date in either plants or other organisms. A database search revealed only 6 characterised protein kinases which shared greater than 30% identities with ATN1 (ranging from 33.5-41.4%) in their catalytic domains; each of the related sequences has mixed serine/threonine and tyrosine kinase affinities. The N-terminal region of ATN1 displays and N-myristoylation motif similar to those found in a number of other protein kinases. Although ATN1 does not belong to an established plant protein kinase subfamily, it is evolutionarily closest to a group which includes kinases involved in transmembrane signalling (the ‘receptor-like kinases’). The ATN1 gene forms part of a small multigene family and is expressed in all organs of the plant studied.


In plants, calcium dependent protein kinases (CDPKs) constitute a unique family of enzymes that is characterized by a C-terminal calmodulin (CaM)-like domain. In this study, we have cloned four partial CDPK cDNAs (CsCDPK1-4) from cucumber by reverse transcription polymerase chain reaction (RT-PCR) using degenerate oligonucleotide primers designed based on conserved regions of the other known CDPKs. Transcript levels of one of the CDPK messengers viz. CsCDPK3 were measured in intact, etiolated,excised cotyledons, hypocotyls and roots following treatments with light or phytohormones (cytokinin/auxin) using a recently evolved high-sensitivity quantitative RT-PCR method (TaqMan analysis). The highest transcript levels of CsCDPK3 were detected in hypocotyls followed by roots and cotyledons. Exposure to light was found to have a down-regulatory effect on CsCDPK3 transcript levels in excised hypocotyls and roots unlike in excised cotyledons where light was found to exert an up-regulatory effect. Treatment with benzyladenine (cytokinin) up-regulated CsCDPK3 transcript levels in cotyledons as opposed to a down-regulatory effect in roots and did not seem to have a significant effect on CsCDPK3 transcript levels in hypocotyls. On the other hand, 2,4-dichlorophenoxyacetic acid (2,4-D) (auxin) treatments did not cause any significant changes in CsCDPK3 transcript levels in hypocotyls, cotyledons or roots. Thus, our results show that light and cytokinin differentially regulate CsCDPK3 transcript levels in a tissue-specific manner.

Most of the important forage and turf grasses are outbreeders, require vernalization to flower, and in some cases are polyploid. With the development of gene sequencing information in grasses, there is an urgent need for a model system to perform large-scale functional analysis of candidate genes. We propose to use Lolium temulentum L. (Darnel ryegrass) as a model system for genetic manipulation studies in forage and turf grasses because L. temulentum has the following advantages: self-fertile, short life cycle (11 weeks), diploid, easy to grow, and closely related with other major grass species. In order to improve tissue culture response of L. temulentum, two relatively responsive lines were crossed and putative hybrid seeds obtained. Analysis of the F1 plants by SSR markers confirmed that the F1 plants were true hybrids. Anthers were dissected from F2 plants of the cross, 3.0% of the cultured anthers responded with the formation of calluses. Green haploid plants were recovered from 48.9% of the anther-derived calluses. Seeds were harvested from doubled haploid plants and mature embryos were used as explants for comparing tissue culture responses with other lines. Besides anther culture, F2 seeds of the cross were subjected to a cycle of selection for callus formation and plant regeneration, and F4 seeds were obtained from the regenerated plants. Comparison of tissue culture response of different lines revealed that the doubled haploid plants had a much higher frequency of embryogenic callus formation than that of the parental lines and the F4 seeds. This is the first report on the generation of green haploid and doubled haploid plants in L. temulentum. The technique was successfully used for the rapid production of homozygous diploid plants that are highly tissue culture responsive. The anther culture-derived new L. temulentum lines could be valuable material for functional test of genes in grasses.


Using degenerated oligonucleotide primers derived from the gibberellin (GA) 20-oxidase sequence from pumpkin (Cucurbita maxima) and Arabidopsis thaliana, an internal fragment of 525 bp was amplified by nested polymerase chain reaction from cDNA of immature inflorescence of perennial ryegrass (Lolium perenne L.). The full-length ryegrass GA 20-oxidase genomic DNA sequence was isolated by genome walking. The deduced full-length cDNA clone (Lp20ox) of perennial ryegrass immature inflorescence was isolated by polymerase chain reaction. Sequence comparison reveals that the putative amino acid sequence shares 57.1% homology with pumpkin (C. maxima) and 92.3% homology with wheat (Triticum aestivum). Expression of Lp20ox in Escherichia coli catalyzed the conversion of GA12- to GA9 and GA53- to GA20, indicating a highly specific GA 20-oxidase activity. Southern blot analysis suggests the presence of two copies of GA 20-oxidase in the ryegrass genome. Lp20ox mRNA accumulated in germinating seeds, expanding leaves, inflorescence, actively growing shoots and internodes, while it could not be detected in developing ovaries and immature seeds.


Summary: GALACTINOL SYNTHASE constitutes a highly homologous, small gene family in maize. ZmGOLS2 cDNA probe detects full-length ZmGOLS transcript in dehydration stressed-, and a smaller transcript (ST) in heat stressed-germinating seeds and callus cells. The ST can be detected in seeds imbibed at temperatures above 30 [deg]C, attaining greatest abundance at 40
At 45°C, the ST is no longer detected and the full-length transcript is again prevalent. Northern blot analysis of poly(A) selected mRNA indicates that the ST is polyadenylated. The ST can be detected by antisense but not by sense RNA probes. Only the five-prime-third of the ZmGOLS2 cDNA is homologous to the ST. However, ribonuclease protection assays (RPA) using a probe to the 5' portion of ZmGOLS2, led to the conclusion that only ZmGOLS3, not ZmGOLS2 transcript, is present in heat stressed seeds. The small transcript detected by ZmGOLS2 probe is not derived from ZmGOLS2 but from an unknown, highly homologous gene. Using 3' RACE, a full-length and a short ZmGOLS3 cDNA were cloned. Sequencing revealed that the short ZmGOLS3 transcript is a fusion of the 5' and 3'-UTR regions of ZmGOLS3. Comparison with the gene sequence revealed that there are no typical intron-exon junction structures around the deleted fragment of ZmGOLS3. Instead, a five base pair, GC-rich sequence delineates the deletion sites used to form the ZmGOLS3 short transcript. Southern blot analysis using maize genomic DNA as a template confirmed that the aberrant ZmGOLS3 transcript was produced due to aberrant RNA processing and is not due to transcription of a ZmGOLS3 pseudogene.


http://www.sciencedirect.com/science/article/B6WPF-47XWK13-5/2/0be58c651e7c2bb57a224b49b0633c38

The lactococcal plasmid pCl658 (58 kb) isolated from Lactococcus lactis ssp. cremoris HO2 encodes the production of a hydrophilic exopolysaccharide (EPS) which consists primarily of galactose and glucuronic acid and which interferes with adsorption of phages o712 and oc2 to cell surface receptors. Examination of the nucleotide sequence of a 21.8-kb region of the plasmid revealed a large genetic cluster consisting of at least 23 putative EPS biosynthetic determinants in addition to the presence of insertion sequences at the 5' and 3' ends. According to homology searches, the genes were organized in specific regions involved in regulation, synthesis and export of the EPS. The predicted products of individual genes exhibited significant homology to exopolysaccharide, capsular polysaccharide (CPS), and lipopolysaccharide (LPS) gene products from a variety of Gram positive and Gram negative bacteria. Evidence of a gene encoding UDP-glucose dehydrogenase is also presented and this is the first description of such a gene in Lactococcus.


http://www.sciencedirect.com/science/article/B6WPF-4D7CD1R-4/2/efc386b2d73bbf5336a68522af16c8a4

The conjugative IncP-9 plasmid pWW0 (TOL) carries transfer genes, many of whose functions can be predicted from sequence similarities to the well-studied IncW and IncP-1 plasmids, and that are clustered with the replication and maintenance genes of the plasmid core. In this study we show that the IncP-9 transfer genes are transcribed from at least three promoter regions. The
promoters for traA and traD act divergently from the region found to encode the origin of transfer, oriT. These promoters regulate expression of traA, B, and perhaps traC in one direction and traD in the other, all of whose gene products are predicted to be involved in relaxasome formation and DNA processing during transfer, and they are repressed by TraA. The third promoter region, upstream of mpfR, is responsible for transcription of mpfR and mpfA to mpfJ, encoding proteins involved in mating pair formation. Transcription from this region is negatively autoregulated by MpfR. Thus the pWW0 transfer genes, like those of the IncP-1 plasmids, are expressed at all times, but kept in control by a negative feedback loop to limit the metabolic burden on the host. Although many of the related mating pair formation systems are, as in pWW0, transcribed divergently from an operon for replication and/or stable inheritance functions, MpfR is not related to the known regulatory proteins of these other transfer systems outside those of the IncP-9 family and indeed the regulators tend to be specific for each plasmid family. This suggests that the general pattern of genetic organisation exhibited by these systems has arisen a number of times independently and must therefore be highly favourable to plasmid survival and spread.


http://www.sciencedirect.com/science/article/B6WPF-47PG7XM-1/2/49a68e02e200a866092744847128eb0a

We report herein the isolation and molecular characterization of pBGR1, the first native plasmid isolated from the genus Bartonella. Cloning and sequencing revealed a 2725-base pair (bp) cryptic plasmid comprising two open reading frames of considerable length, which were designated rep and mob. The regions containing rep and mob are separated by 140-bp inverted repeat sequences and display a difference in G + C content from one another. A 1435-bp SacI-BclI fragment containing the rep gene is sufficient to mediate replication in the species Bartonella henselae and Bartonella tribocorum, while this replicon does not appear to be functional in Escherichia coli. The Rep protein of 190 amino acids (aa) shares homology to putative replication proteins of cryptic plasmids of Gram-negative origin, which form a subgroup of the rolling-circle replication proteins of the pSN2 plasmid superfamily of Gram-positive bacteria. The Mob protein of 333 aa is related to mobilization proteins of several cryptic plasmids and is associated with a conserved recombination site A. The tra functions of RP4 can mobilize pBGR1 derivatives in a mob-dependent manner. Mobilizable pBGR1-based E. coli-Bartonella spp. shuttle vectors were constructed and were shown to be maintained in B. tribocorum during in vivo passage in a rat model in the absence of antibiotic selection. The small size and stability of these shuttle cloning vectors should render them particularly valuable for genetic studies in Bartonella spp.

Postgrad. Med. J. (1)


http://pmj.bmjournals.com/cgi/content/abstract/80/940/107

Drug metabolism may be perturbed by genetically determined differences in the metabolic activity
of cytochrome P450 enzymes. The authors encountered extensive bleeding in a patient receiving warfarin for anticoagulation after the introduction of celecoxib, an anti-inflammatory drug. As the CYP2C9 enzyme metabolises these drugs, it was determined whether variant alleles were responsible for altering warfarin handling. Genetic analysis established that the patient was a compound heterozygote with CYP2C9*2 and *3 variant alleles, which exhibit lower drug metabolising capacity and enhance susceptibility to drug toxicity.

**Postharvest Biology and Technology (3)**


Penicillium roqueforti was recently reclassified into the three species *P. roqueforti*, *Penicillium carneum*, and *Penicillium paneum* based on differences in ribosomal DNA sequences and secondary metabolites, e.g. mycotoxins. This is the first report on interaction between these closely related mould species under stress conditions. The yeast *Pichia anomala* (J121) inhibits growth of *P. roqueforti* in grain stored in malfunctioning airtight storage systems. The ability of *P. anomala* to inhibit all three species of the *P. roqueforti* group was examined in separate experiments as well as the competition between the three mould species when co-cultured with or without the yeast in non-sterile wheat grain (aw 0.95) under restricted air supply. Mould growth was analysed by dilution plating after 14 days and the individual colonies identified by random amplified polymorphic DNA (RAPD) fingerprinting. When co-culturing the *P. roqueforti* group in wheat with the yeast all three species were able to grow to the same extent. Also, when co-culturing all species of the *P. roqueforti* group together with *P. anomala*, the growth response of the three species was very similar. At yeast levels of 104 CFU g⁻¹ grain a pronounced inhibition was observed and at 105 CFU g⁻¹ grain a fungicidal effect was detected, indicating a potentiated effect of *P. anomala* when co-culturing the three mould species.


http://www.sciencedirect.com/science/article/B6TBJ-4FG899S-1/2/b3d3f06e934f3144d24284b14c539946

A cDNA encoding a putative ethylene receptor (DG-ERS1) was isolated from chrysanthemum [Dendranthema grandiflorum (Ramat.) Kitamura] using a combination of reverse transcription PCR (RT-PCR), cDNA library screening and 5'-RACE techniques. The cDNA (2427 bp) contained an open reading frame of 1920 bp coding for 640 amino acids. The predicted DG-ERS1 protein has an amino-terminal ethylene sensor domain and a histidine kinase domain, but lacks a receiver domain. The DG-ERS1 protein has 72, 70 and 69% similarity to Arabidopsis ERS1, tomato *Never ripe* (NR) and carnation DC-ERS2, respectively. Real time PCR analysis revealed that DG-ERS1 mRNA was present in a large amount in ligulate corollas (hereafter, petals for
short) and mature leaves of an ethylene-sensitive cultivar 'Seiko-no-makoto' at the full-opening stage of the flower, and the amount decreased with time or in response to a 12-h ethylene treatment. In an ethylene-insensitive cultivar 'Iwa-no-hakusen', the amount of DG-ERS1 mRNA in petals was one-fourth and that in mature leaves was only one-twentieth of the amount in 'Seiko-no-makoto' at the full-opening stage, and its amount in both tissues scarcely changed with time or in response to a 12-h ethylene treatment. These findings suggest the involvement of DG-ERS1 in the perception of ethylene in cut chrysanthemum plants, especially in those of 'Seiko-no-makoto' cultivar.


http://www.sciencedirect.com/science/article/B6TBJ-46Y5881-2/2/6725fa548dc14d77ba35be949858af0

Two distinct clones having high nucleotide identity to the sequences encoding ascorbate peroxidase (APX) were isolated from broccoli (Brassica oleracea L. var. italica). Deduced amino acid sequences of both cDNAs, BO-APX 1 (accession number AB078599) and BO-APX 2 (accession number AB078600), shared identity of 92.8% and there was more than 80% identity between BO-APXs and other plant cytosolic APXs at the protein level. Gene expression and protein levels of BO-APX 1 and BO-APX 2 were investigated in various parts of broccoli after harvest. Transcript levels of BO-APX 2 gradually increased in florets, while those of BO-APX 1 decreased in florets after harvest. BO-APX 1 and BO-APX 2 were expressed in Escherichia coli as a fusion protein with glutathione S-transferase (GST) and purified to homogeneity by glutathione sepharose 4B column chromatography. Both proteins of BO-APX 1 and BO-APX 2 appeared as a single major band on SDS-PAGE corresponding to a mass of 25 kDa and reacted with polyclonal antibodies raised against recombinant BO-APX 1. Both enzymes showed high specificities for ascorbate and hydrogen peroxide. The km values of recombinant BO-APX 1 and BO-APX 2 for ascorbate were 395 and 526 [μM] and those for hydrogen peroxide were 15 and 7 [μM], respectively. The role of APX was discussed in relation to ascorbate breakdown in broccoli florets during senescence.

Preventive Veterinary Medicine (1)


http://www.sciencedirect.com/science/article/B6TBK-45S9CHT-1/2/f32a690a77e9483b44dc84637601db16

The spacer oligonucleotide typing (spoligotyping) method was used to differentiate 62 Mycobacterium bovis isolates obtained from tissues with macroscopic lesions typical of tuberculosis in dairy cattle from different regions of Mexico. Our purpose was to see if a strain from one region was genetically different from those of other regions (with the long-term aim of doing molecular trace back of isolates obtained in the laboratory). Results from the genetic analysis indicate that M. bovis isolates cannot be grouped by geographic location due to a wide
range of genetic types involved in dairy cattle infections. Isolates even from the same herd showed different spoligotypes but some isolates from different region had similar genetic patterns. Genetic typing without epidemiologic information does not seem to be a plausible method to trace back animals to source of origin to detect and eliminate sources of infection.

**Process Biochemistry (1)**


http://www.sciencedirect.com/science/article/B6THB-49PRGPT-1/2/877fa90fcfb9125ab47a7d47e99aa553

A gene, tgB1, encoding transglutaminase (TGase) in Streptoverticillium ladakanum B1 was cloned and expressed in Streptomyces lividans. The tgB1 gene consisted of an open reading frame of 1230 nucleotides encoding a protein of 410 amino acids with a calculated molecular weight of 45780 Da. The deduced amino acid sequence is highly homologous to TGases from Streptoverticillium spp. but exhibits little homology with TGases of Bacillus subtilis and mammalian origins. The putative active site, YGCVG, conserved in Streptoverticillium TGases is also present in TgB1. No -10 and -35 regions of the putative promoter could be identified. Two A+T-rich regions, characteristics of a promoter sequence, were found at bp 238-269 and bp 631-681. The tgB1 gene was expressed in S. lividans JT46 under the control of its endogenous promoter. Immunoblotting of SDS-PAGE revealed that, in addition to protein bands with sizes corresponding to those of the unprocessed and mature TgB1, several bands with sizes in between reacting with anti-TgB1 IgG were present in the culture supernatant of the recombinant strain. This suggests that the recombinant TgB1 was not correctly processed during secretion in the transformed S. lividans JT46.

**Progress in Neuro-Psychopharmacology and Biological Psychiatry (3)**


http://www.sciencedirect.com/science/article/B6TBR-480CSPJ-3/2/c9854359f74ce9fa0edf4ac819445cb8

Previous studies in crosses between the C57BL/6J (B6) and the DBA/2J (D2) mice have implicated a role of the genes encoding for the 67- and 65-kDa isoforms of the glutamate decarboxylase (Gad1 and Gad2) in the manifestation and severity of multiple ethanol-related traits such as acute ethanol withdrawal severity [Buck, K.J., Metten, P., Belknap, J.K., Crabbe, J.C., 1997. Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. J. Neurosci. 17, 3946-3955], ethanol preference [Phillips, T.J., Belknap, J.K., Buck, K.J.,...
Cunningham, C.L., 1998. Genes on mouse chromosomes 2 and 9 determine variation in ethanol consumption. Mamm. Genome 9, 936-941 and ethanol-induced locomotion [Demarest, K., McCaughran Jr., J., Mahjubi, E., Cipp, L., Hitzemann, R., 1999. Identification of an acute ethanol response quantitative trait locus on mouse chromosome 2. J. Neurosci. 19, 549-561]. Strain-specific sequencing experiments as well as gene expression studies in drug-naive and ethanol-treated D2 and B6 mice were carried out. The Gad1 sequence was similar, the Gad2 cDNA carried only a silent polymorphism (1017 G>C) between both strains. In addition, no significant GAD65 or GAD67 expression differences were detected in either drug-naive or acute ethanol withdrawn animals by Western blot experiments. Therefore, these results do not support the hypothesis of an involvement of Gad1 or Gad2 in the pathophysiology of acute ethanol withdrawal severity and the other ethanol related traits.


http://www.sciencedirect.com/science/article/B6TBR-43S179D-1/2/a7702e7b3755f3805b44ba7575ac8cfb

1. Alterations in the serotonergic neurotransmission have been frequently described for patients suffering from alcoholism, anxiety disorders and narcolepsy. 2. The authors tested for association of the 5-HT2A receptor polymorphism (T102C) and the intron 7 tryptophan hydroxylase (TPH) polymorphism (A218C) among 176 alcohol dependent patients, 35 patients with panic disorder, 50 patients with generalized anxiety disorder, 55 patients with narcolepsy and 87 healthy controls. 3. Allele and genotype frequencies of the 5-HT2A receptor polymorphism (T102C), the intron 7 TPH polymorphism (A218C) were almost similar between the patients suffering from alcohol dependence, panic disorder, generalized anxiety disorder and narcolepsy. 4. There was no association between the 5-HT2A receptor polymorphism (T102C), the intron 7 TPH (A218C) polymorphisms and alcohol dependence, panic disorder, generalized anxiety disorder and narcolepsy in our subsets of German patients.


http://www.sciencedirect.com/science/article/B6TBR-3XP0K3F-C/2/c84f91b9a59f34154f3885895ee8fd7

1. 1. Full-length form of human presenilin 1 (PS1) is processed and an N-terminal fragment (28 KD) and C-terminal fragment (19 KD) are generated. To elucidate the possible role of presenilin mutations in Alzheimer's disease (AD), the authors analyze the effects of AD-linked mutations on PS1 processing in cultured cells. 2. 2. Complementary DNAs encoding genes for human PS1 harboring twenty-nine missense mutations linked with familial Alzheimer's disease (FAD) were introduced into PC12 cells. Human PS1 exogenously expressed in the cells was detected by immunoblotting using a monoclonal antibody that recognized the N-terminal region of human PS1. The amounts of full-length form (48 KD) and N-terminal fragment (28 KD) of PS1 was quantified by densitometrical analysis. 3. 3. The ratio of the N-terminal fragment to total PS1 was reduced by twenty-nine mutations. The specific effects on PS1 processing varied according to mutation. 4. 4. These results suggest that AD-linked missense mutations of PS1 are involved in neurodegeneration via inhibition of PS1 processing.
Neuronal development and differentiation require a variety of cell interactions. Diffusible molecules from target neurons play an important part in mediating such interactions. Our early studies used explant culture technique to examine the factors that enhance the differentiation of septo-hippocampal cholinergic neurons, and they revealed that several components resident in the hippocampus are involved in the differentiation of presynaptic cholinergic neurons in the medial septal nucleus. One of these components, originally purified from young rat hippocampus, is a novel undecapeptide (hippocampal cholinergic neurostimulating peptide; HCNP); this enhances the production of ChAT, but not of AchE. Later experiments revealed that: (1) a specific receptor appears to mediate this effect; (2) NGF and HCNP act cooperatively to regulate cholinergic phenotype development in the medial septal nucleus in culture; and (3) these two molecules differ both in their mechanism of release from the hippocampus and their mechanism of action on cholinergic neurons. The amino acid sequence deduced from base sequence analysis of cloned HCNP-precursor protein cDNA shows that HCNP is located at the N-terminal domain of its precursor protein. The 21 kDa HCNP precursor protein shows homology with other proteins, and it functions not only as an HCNP precursor, but also as a binding protein for ATP, opioids and phosphatidylethanolamine. The distribution and localization of HCNP-related components and the expression of their mRNAs support the notion that the precursor protein is multifunctional. In keeping with its multiple functions, the multiple enhancers and promoters found in the genomic DNA for HCNP precursor protein may be involved in the regulation of its gene in a variety of cells and at different stages of development. Furthermore, several lines of evidence obtained from studies of humans and animal models suggest that certain types of memory and learning disorders are associated with abnormal accumulation and expression of HCNP analogue peptide and/or its precursor protein mRNA in the hippocampus.

Prostaglandines and Medicine (1)


http://www.sciencedirect.com/science/article/B7GHB-4BWX56-1/2/8487491a2a9345060e91468a7b52ed20

Twelve patients underwent induction of labor at term, seven by prostaglandin F2[alpha] (PGF) and five by oxytocin infusion. Serum samples were drawn before induction and at two hour intervals after infusion was started, throughout labor. The samples were analyzed for estriol (E3)
and its principal conjugates, estriol-3-sulfate (E3-3S), estriol-16-glucosiduronate (E3-16G), estriol-3-glucosiduronate (E3-3G), and estriol-3-sulfate-16-glucosiduronate (E3-SG). No significant changes occurred before eight hours of infusion. Two pre-eclamptic patients showed a 100[deg] increase in serum E3 while receiving PGF. The increases correlated best with rising levels of E3-SG. None of the five pre-eclamptic patients given oxytocin showed this striking change. These observations raise the possibility that PGF may alter the metabolism of estriol in pre-eclamptic patients, during labor.

**Prostaglandins** (2)


http://www.sciencedirect.com/science/article/B7CRH-4F5T6BT-3/2/517257ef4b7f1c387ee97b67a9a117e8

Leukotrienes (LTs), the 5-lipoxygenase (5-LOX) metabolites of arachidonic acid, have roles in many biological processes relevant to the gastrointestinal tract, including intestinal inflammation. We screened two well-known human intestinal epithelial cell lines, HT29 and Caco-2, for evidence of LT-associated enzyme transcripts and LT synthesis. Northern blot analysis of total RNA from both intestinal lines demonstrated high levels of transcripts for LTA4 hydrolase, a multisubstrate enzyme that converts the 5-LOX metabolite, LTA4, to LTB4. With total RNA, the 5-LOX transcript was detected only in HT29. Caco-2 failed to show 5-LOX message even with poly A-containing RNA, although the transcript could be amplified with the polymerase chain reaction. Messenger RNA for FLAP, the 5-lipoxygenase-activating protein, was detectable in both cell lines, but only with poly A-containing RNA. In a sonicated cell preparation, HT29, but not Caco-2, revealed detectable levels of 5-HETE and LTB4. These results suggest that certain intestinal epithelial cells possess a limited capacity to synthesize LTs.


http://www.sciencedirect.com/science/article/B7CRH-4F66YRD-R/2/df271feeab83aadfc43dab3e557f48a5

This paper reports on the involvement of c-MYB in the regulation of 5-lipoxygenase gene expression during differentiation of human HL-60 cells. We demonstrate that c-MYB binds, the 5-lipoxygenase promoter in undifferentiated cells but not in DMSQ-diff erentiated cells. Also, we show that overexpression of c-myb cDNA in differentiated HL-60 cells represses the 5-lipoxygenase gene expression.

**Prostaglandins & Other Lipid Mediators** (4)
We examined and compared the in vitro effects of misoprostol (synthetic prostaglandin E1 (PGE1) analogue) on prostaglandin E2 (PGE2) secretion and EP3 receptor mRNA expression in the pregnant rat myometrium and cervix at 19 days gestation. Myometrial and cervical tissue samples were exposed to media with or without misoprostol (50 or 100 pg/ml) and incubated for 15 and 30 min, and 1, 3, 6, 12, and 24 h. Media and tissue samples were collected for quantification of PGE2 and mRNA expression of rEP3[alpha] and rEP3[beta] receptor, respectively. PGE2 secretion increased following exposure to the 100 pg/ml dose only. In the myometrium, 50 and 100 pg/ml misoprostol induced elevations in rEP3[alpha] and rEP3[beta] receptor mRNA expression. rEP3[alpha] and rEP3[beta] receptor mRNA expression in the cervix was not different from controls. These data demonstrate that the EP3 receptor is differentially expressed in the myometrium and cervix in response to misoprostol. This may account for the ability of misoprostol to stimulate the myometrium when administered for cervical ripening.

Recent studies indicate that the corpus luteum (CL) may be a source of prostaglandin F2[alpha] (PGF2[alpha]) for regression. We investigated expression of mRNA and protein for prostaglandin G/H synthase (PGHS) in the CL of immature superovulated rats following administration of PGF2[alpha]. We observed an increase in mRNA for PGHS-2, the induced isoform, at 1 h and protein at 8 and 24 h after treatment. One hour after PGF2[alpha], there was also a progressive decrease in plasma progesterone concentration. There were no changes, however, in expression of PGHS-1, the constitutive isoform, over the 24 h sampling period. These results indicate that PGHS-2 increases following PGF2[alpha] treatment and that expression of this enzyme in the rat CL may contribute to the luteolytic mechanism.

To examine the biochemical regulation of morphine sulfate (MS) on prostanoid synthesis, conscious newborn piglets received a bolus dose of 100 [mu]g/kg followed by a continuous infusion dose of 100 [mu]g/kg/h. The control group received equivalent volume bolus and continuous infusion of 5% dextrose. Blood samples were drawn from the femoral artery and
sagittal sinus vein before, during and after infusion for measurement of prostanoids. The expression of mRNAs encoding cyclooxygenases (COX)-1 and -2 in the brainstem, thalamus, cortex, and cerebellum of the newborn piglets were also examined. Systemic PGE2 levels declined substantially during and post MS infusion (p 2 levels increased following the bolus dose (p p 1[alpha] levels, however, in the cerebral circulation 6-ketoPGF1[alpha] levels increased 146% (p 2 levels increased transiently at 4 h (p 2 increased at 0.5 and 1 h (p p p < 0.001) increased expression of COX-1 mRNA in the MS-infused brain samples. In contrast, no differences in COX-2 mRNA were detected between the groups. These data imply that MS may have significant effects on prostanoid synthesis in the newborn. The data further show that the MS-induced prostanoid responses appear to be mediated via COX-1.


http://www.sciencedirect.com/science/article/B6T3H-433NRKR-3/2/7ce4a187680dca48629c3903cf5f2223

The objective of the present study was to evaluate second messenger regulation of prostaglandin synthase-2 (PGS-2) mRNA and PGS-2 promoter in ovine granulosa cells and large luteal cells. In granulosa cells, PGS-2 mRNA was induced by forskolin (PKA activator) but not by phorbol didecanoate (PDD; PKC activator) with maximal stimulation at 24 h. In contrast, PDD was the more potent inducer in large luteal cells with the most dramatic effect by 4 h. Similarly, forskolin but not PDD increased media PGF2[alpha] in granulosa cells at 24 h; whereas, PDD but not forskolin increased PGF2[alpha] at 4 h and 24 h in large luteal cells. To evaluate if these effects were due to activation of transcription, a plasmid was constructed containing 1500 bp of PGS-2 promoter linked to a luciferase gene. Forskolin stimulated transcription from this construct in granulosa cells (5-fold); whereas, PDD but not forskolin stimulated transcription in large luteal cells (40-fold). Taking these findings together, we propose that transcriptional regulation of the PGS-2 gene changes from PKA-dependence in granulosa cells to PKC-dependence in large luteal cells after luteinization.

Prostaglandins & Other Lipid Mediators  (1)


http://www.sciencedirect.com/science/article/B6T3H-4DVW1XX-2/2/7aa6b94a89af70d65e038657b00773bb

The predominating prostaglandins of human seminal fluid are 19R-hydroxyprostaglandins E1 and E2, conceivably formed sequentially by prostaglandin H (PGH) synthase-2, PGH 19-hydroxylase (CYP4F8), and microsomal PGE synthase-1 of seminal vesicles. Our aim was to study this enzyme system. Quantification by real-time PCR suggested that the transcripts of PGH synthase-2, CYP4F8, and microsomal PGE synthase-1 were abundant and correlated in seminal vesicles.
of seven patients (p n-2 hydroxylation of PGH1 and PGH2 and n-3 hydroxylation of arachidonic acid. Arachidonic acid was oxidized to 18-hydroxyarachidonic acid and to PGE2 and by microsomes of seminal vesicles in the presence of NADPH and GSH, and to relatively small amounts of 19-hydroxy-PGE2. We conclude that PGH synthase-2, CYP4F8, and PGE synthase-1 likely forms 19-hydroxy-PGE compounds in seminal vesicles and vas deferens, but the catalytic properties of CYP4F8 suggest additional biological functions. Recombinant CYP4F8 was also found to catalyze n-2 hydroxylation of PGI2 and carbaprostacyclin (Km [not, vert, similar] 40 [mu]M), and n-2 and n-3 hydroxylation of carbocyclic TXA2.

Protein Eng.  (1)


http://peds.oupjournals.org/cgi/content/abstract/15/3/233

A three-dimensional model of the variable domain of the atrazine-specific Fab fragment K411B was constructed by molecular modeling using known structures of highly homologous immunoglobulins as templates. Molecular dynamic simulations and cross-reactivity data were used to predict residues responsible for the binding of the hapten 4-chloro-6-(isopropylamino)-1,3,5-triazine-2-(6-aminohexanecarboxylic acid) (iPr/Cl/C6) instead of atrazine. Specific binding pockets could be defined for the chlorine, the isopropylamino group and the C6-spacer of the hapten. The influence of various amino acids on hapten binding was investigated by site-directed mutagenesis, and the effect of these mutations was analyzed by capture ELISA using the hapten iPr/Cl/C6 and 4-amino-6-chloro-1,3,5-triazine-2-(6-aminohexanecarboxylic acid) (H/Cl/C6). GlyH100a seems to be important in determining the conformation of the heavy-chain complementarity determining region H3; replacing it with any other residue prevented the binding of the hapten. Altering residues responsible for the binding of the chlorine atom (TrpH33, GluH50 and TyrL96) decreased the affinity significantly. Hapten-spacer recognition can be attributed to the interaction with PheL32; replacing PheL32 by leucine reduced the affinity towards iPr/Cl/C6. A triple mutant Fab fragment (GlnL89Glu, ValH37Ile and GluL3Val) showed an affinity 5-fold greater towards iPr/Cl/C6 compared to the wild-type K411B, as a result of better recognition of the isopropylamino group of iPr/Cl/C6.

Protein Eng. Des. Sel.  (2)


http://peds.oupjournals.org/cgi/content/abstract/17/6/517

Recent research on the flavoenzyme D-amino acid oxidase from Rhodotorula gracilis (RgDAAO)
has revealed new, intriguing properties of this catalyst and offers novel biotechnological applications. Among them, the reaction of RgDAAO has been exploited in the analytical determination of the D-amino acid content in biological samples. However, because the enzyme does not oxidize acidic D-amino acids, it cannot be used to detect the total amount of D-amino acids. We now present the results obtained using a random mutagenesis approach to produce RgDAAO mutants with a broader substrate specificity. The libraries of RgDAAO mutants were generated by error-prone PCR, expressed in BL21(DE3)pLysS Escherichia coli cells and screened for their ability to oxidize different substrates by means of an activity assay. Five random mutants that have a modified substrate specificity, more useful for the analytical determination of the entire content of D-amino acids than wild-type RgDAAO, have been isolated. With the only exception of Y223 and G199, none of the effective amino acid substitutions lie in segments predicted to interact directly with the bound substrate. The substitutions appear to cluster on the protein surface: it would not have been possible to predict that these substitutions would enhance DAAO activity. We can only conclude that these substitutions synergistically generate small structural changes that affect the dynamics and/or stability of the protein in a way that enhances substrate binding or subsequently catalytic turnover.


http://peds.oupjournals.org/cgi/content/abstract/17/4/341

We tested whether it is possible to alter the substrate specificity of cholesterol oxidase for similarly sized sterols, i.e. cholesterol, \{beta\}-sitosterol and stigmasterol. Using existing X-ray crystal structures, we made a model of the predicted Michaelis complex of cholesterol and cholesterol oxidase. Based on this model, we identified five residues that are in direct contact with the steroid tail, Met58, Leu82, Val85, Met365 and Phe433. We prepared seven mutant libraries that contained the codon NYS (N = A, C, G, T; Y = C, Y; S = C, G) at one, two or three of the targeted positions by cassette mutagenesis. The libraries were screened for catalytic activity against three different sterols under kcat*/Km* conditions with 25 mol% sterol/DOPC unilamellar vesicles. The results of our screens suggest that specific packing interactions are not realized in the transition state of binding and that loss of active site water may be the predominant source of binding energy.

Protein Expression and Purification (8)


http://www.sciencedirect.com/science/article/B6WPJ-48H87K9-1/2/9864d2d5389c9c899c4ef1ff5f0a8c8

Until recently, glycosylation of proteins in prokaryotes was regarded as uncommon and thought to be limited to special cases such as S-layer proteins and some archaean outer membrane proteins. Now, there are an increasing number of reports of bacterial proteins that are glycosylated. Pilin of pathogenic Neisseria is one of the best characterised post-translationally modified bacterial
proteins, with four different types of modifications reported, including a novel glycosylation. Pilin monomers assemble to form pilus fibres, which are long protein filaments that protrude from the surface of bacterial cells and are key virulence factors. To aid in the investigation of these modifications, pure pilin is required. A number of pilin purification methods have been published, but none are appropriate for the routine purification of pilin from many different isolates. This study describes a novel, rapid, and simple method of pilin purification from Neisseria meningitidis C311#3, which facilitates the production of consistent quantities of pure, native pilin. A 6 x histidine tag was fused to the C-terminus of the pilin subunit structural gene, pilE, via homologous recombination placing the 6 x histidine-tagged allele in the chromosome of N. meningitidis C311#3. Pilin was purified under non-denaturing conditions via a two-step process using immobilised metal affinity chromatography (IMAC), followed by dye affinity chromatography. Analysis of the purified pilin confirmed that it retained both of the post-translational modifications examined. This novel approach may prove to be a generally applicable method for purification and analysis of post-translationally modified proteins in bacteria.


http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-C/2/f121889f8444bc6f8d3e224f9840af40

The ospA gene of Borrelia burgdorferi encodes an outer membrane protein which is a major antigen of the Lyme disease agent. Two sequence-specific sets of oligonucleotide primers were used to specify the amplification of the ospA coding sequence by the polymerase chain reaction. One set allowed the entire ospA sequence to be amplified, while the other primed amplification of a truncated form of ospA lacking the first 17 codons specified by the wild-type ospA structural gene, residues believed to constitute a signal sequence which normally would direct localization of the ospA protein to the Borrelia cell's outer membrane. Each set of primers also contained sequences near their 5' ends which facilitated cloning of the amplified DNA directly into a high level expression system based on bacteriophage T7 genetic elements. We showed that the fulllength OspA protein is synthesized poorly in Escherichia coli and it is associated with the insoluble membrane fraction. In contrast, the truncated form can be expressed to very high levels and it is soluble. The truncated protein was purified to homogeneity and partially characterized. Its N-terminal sequence and molecular weight derived from sodium dodecyl sulfate-polyacrylamide gel electrophoresis agree with those deduced from the DNA sequence. It is a monomer with a native molecular weight of 28,000 and it is very resistant to digestion by trypsin even though it is rather rich in lysine residues (16 mol%). Recombinant OspA protein synthesized in E. coli is recognized by antibodies in sera of Lyme patients, which suggests that the protein may be useful in immunoassays and as a possible immunogen to protect against Lyme borreliosis.


http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-30/2/14a890b8d70c4404e1c3153521eff833

Rat liver protein disulfide isomerase (PDI) catalyzes the oxidative folding of proteins containing disulfide bonds. We have developed an efficient method for its overproduction in Escherichia coli. Using a T7 RNA polymerase expression system, isolated yields of 15-30 mg/liter of recombinant rat PDI are readily obtained. Convenient purification of the enzyme from E. coli lysates involves
ion-exchange (DEAE) chromatography combined with zinc chelate chromatography. The recombinant PDI shows catalytic activity identical to that of PDI isolated from bovine liver in both the reduction of insulin and the oxidative folding of ribonuclease A. The enzyme is expressed in E. coli as a soluble, cytoplasmic protein. After complete reduction and denaturation in 6 guanidinium hydrochloride, PDI regains complete activity within 3 min after removal of the denaturant, implying that disulfide bonds are not essential for the maintenance of PDI tertiary structure. Both the protein isolated from E. coli and the protein isolated from liver contained free cysteine residues (1.8 +/- 0.2 and 1.4 +/- 0.3 SH/monomer, respectively).


http://www.sciencedirect.com/science/article/B6WPJ-4DW8PPM-1/2/76ad2abb28f4b11bc311a7610a0a11e3

The type III secretion system (YscC) protein of Yersinia pestis plays an essential role in the translocation of Yersinia outer proteins (Yops) into eukaryotic target cells through a type III secretion mechanism. To assess the immunogenicity and potential protective efficacy of YscC against lethal plague challenge, we cloned, overexpressed, and purified YscC using two different bacterial expression and purification systems. The resulting expression plasmids for YscC, pETBlue-2-YscC and pTYB11-YscC, were regulated by robust T7 promoters that were induced with isopropyl-\[\beta\]-d-thiogalactopyranoside. The intein-fusion pTYB11-YscC system and the six-histidine-tagging pETBlue-2-YscC system were both successful for producing and purifying YscC. The intein-mediated purification system produced about 1 mg of soluble YscC per liter of bacterial culture while the YscC-His6-tag method resulted in 16 mg of insoluble YscC per liter of bacterial culture. Protein identity for purified YscC-His6 was confirmed by ion trap mass spectrometry. Antisera were produced against both YscC and YscC-His6. The specific immune response generated in YscC-vaccinated mice was relative to the particular purified protein, YscC or YscC-His6, which was used for vaccination as determined by Western blot analysis and ELISA. Regardless of the purification method, either form of the YscC protein failed to elicit a protective immune response against lethal plague challenge with either F1 capsule forming Y. pestis CO92 or the isogenic F1- Y. pestis C12.


http://www.sciencedirect.com/science/article/B6WPJ-47731DH-5/2/dfd306c22eb5ff0bf43054e6e08b5a4a


http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-T/2/59cedf2018b50bd02a7113e7c836827a

The most frequently occurring kringle 4 domain of human apolipoprotein (a), Kringle 4-subtype 2 (K42), was expressed as a fusion protein with the maltose binding protein in Escherichia coli using the "tac" promoter. Although the fusion protein was expressed without a signal sequence,
25% was secreted into the periplasmic space; the remainder was found associated with the soluble cytosolic fraction. The fusion protein was readily isolated from whole cell lysate by amylose agarose affinity chromatography. Although a factor Xa cleavage site was engineered into the fusion protein, it was found that release of the K42 protein was most conveniently achieved by proteolysis with subtilisin A. The cleavage product produced in this way was shown to be intact K42 with only the first three amino acid residues of the leading flanking peptide missing, as judged by N-terminal sequence analysis. K42 was isolated from the hydrolysate by FPLC on a Mono-Q column with a yield of 170 +/- 30 [mu]g/g wet cells. The resulting protein was monomeric in phosphate-buffered saline as judged by size-exclusion chromatography and appeared to be folded as shown by spectroscopic and immunological assays. The recombinant K42 did not bind to either lysine- or proline-Sepharose, suggesting that the ligand binding activities of lipoprotein (a) may reside in the other kringle domains of apolipoprotein (a).


We compared the expression and degradation of three cloned malarial proteins in a pair of isogeneic strains of Escherichia coli that differed at the htpR locus. The htpR locus encodes an alternate [sigma] factor necessary for the transcription of heat shock promoters. Plasmodium sequences were cloned from polymerase chain reaction-amplified DNA initiated by oligonucleotide primers that were specific for the gene coding regions to be expressed. The amplified DNA was cloned and expressed in a vector that encodes a strong T7 promoter and translation-initiation signal. The total cell yield of two of the expressed proteins was found to be increased when synthesis occurred in a E. coli htpR mutant. Pulse-chase experiments showed that the increased protein yield correlated with a decrease in the degradation of the protein in the htpR strain. A two- to seven-fold increase in the half-life of the malaria proteins was observed in the E. coli htpR- background as compared to htpR+. We found no difference in survival of the E. coli K165 htpR mutant and isogeneic parent during thermal induction. Since the synthesis of the heat shock [sigma] factor did not significantly influence survival of E. coli and htpR expression results in increased degradation of foreign proteins, the E. coli htpR mutant was a valuable host strain for production of foreign proteins.

ADP-ribosyltransferases including toxins secreted by Vibrio cholera, Pseudomonas aeruginosa, and other pathogenic bacteria inactivate the function of human target proteins by attaching ADP-ribose onto a critical amino acid residue. Cross-species polymerase chain reaction (PCR) and database mining identified the orthologs of these ADP-ribosylating toxins in humans and the mouse. The human genome contains four functional toxin-related ADP-ribosyltransferase genes (ARTs) and two related intron-containing pseudogenes; the mouse has six functional orthologs. The human and mouse ART genes map to chromosomal regions with conserved linkage synteny. The individual ART genes reveal highly restricted expression patterns, which are largely conserved in humans and the mouse. We confirmed the predicted extracellular location of the ART proteins by expressing recombinant ARTs in insect cells. Two human and four mouse ARTs contain the active site motif (R-S-EXE) typical of arginine-specific ADP-ribosyltransferases and exhibit the predicted enzyme activities. Two other human ARTs and their murine orthologues deviate in the active site motif and lack detectable enzyme activity. Conceivably, these ARTs may have acquired a new specificity or function. The position-sensitive iterative database search program PSI-BLAST connected the mammalian ARTs with most known bacterial ADP-ribosylating toxins. In contrast, no related open reading frames occur in the four completed genomes of lower eucaryotes (yeast, worm, fly, and mustard weed). Interestingly, these organisms also lack genes for ADP-ribosylhydrolases, the enzymes that reverse protein ADP-ribosylation. This suggests that the two enzyme families that catalyze reversible mono-ADP-ribosylation either were lost from the genomes of these nonchordata eucaryotes or were subject to horizontal gene transfer between kingdoms.


We describe expression, purification, and characterization of three site-specific mutants of recombinant human stefin B: H75W, P36G, and P79S. The far- and near-UV CD spectra have shown that they have similar secondary and tertiary structures to the parent protein. The elution on gel-filtration suggests that recombinant human stefin B and the P36G variant are predominantly monomers, whereas the P79S variant is a dimer. ANS dye binding, reflecting exposed hydrophobic patches, is highest for the P36G variant, both at pH 5 and 3. ANS dye binding also is increased for stefin B and the other two variants at pH 3. Under the chosen conditions the highest tendency to form amyloid fibrils has been shown for the recombinant human stefin B. The P79S variant demonstrates a longer lag phase and a lower rate of fibril formation, while the P36G variant is most prone to amorphous aggregation. This was demonstrated by ThT fluorescence as a function of time and by transmission electron microscopy.

Psychiatry Research (5)
Social phobia, particularly the generalized form, is strongly familial and frequently comorbid with major depression, panic disorder, and obsessive-compulsive disorder. It has also recently been shown to be responsive to selective serotonin reuptake inhibitors. We conducted a study to determine if generalized social phobia is genetically linked to either of two candidate genes: the serotonin transporter protein (5HTT) gene, or the 5HT2A receptor (5HT2AR) gene. Rates of social phobia (using several phenotype definitions) were ascertained and blood samples obtained from consenting first-degree family members of generalized social phobic probands. 5HT2AR and 5HTT genotyping was performed using the polymerase chain reaction (PCR). Linkage was tested using LINKAGE and GENEHUNTER software. No evidence of linkage was found; power analysis indicated that failure to find linkage was unlikely due to inadequate statistical power. These findings reasonably exclude linkage between generalized social phobia and the 5HTT or 5HT2AR genes in these samples, although modifier effects cannot be ruled out. Other 5HT receptor subtypes or indirect modulatory effects of 5HT on other neurotransmitter systems may be involved.

The HTR1B receptor gene has been linked to antisocial alcoholism in a Finnish population and an American Indian tribe [Lappalainen et al., Arch. Gen. Psychiatry, 55 (1998) 989]. Using a candidate gene approach, we genotyped 209 patients with alcoholism, 108 patients with major depression, 32 patients with panic disorder, 50 patients with generalized anxiety disorder, 58 patients with narcolepsy and 74 healthy volunteers for the HTR1B 861G>C polymorphism. There was a higher frequency of the HTR1B 861G alleles among the alcohol-dependent patients as compared to the control subjects ([χ²]=4.02, d.f.=2, P=0.04). However, the association resulted from higher frequencies of the opposite alleles (HTR1B 861G), as originally reported by Lappalainen et al. (1998). Although the association in our study might be due to a type I error, the higher degree of HTR1B allele sharing within both populations could also argue for another alcoholism-relevant gene within the proximity of the HTR1B gene on human chromosome 6.

Epidemiological data and family studies in schizophrenia show that genetic factors contribute to the vulnerability to this disorder. The homeogene Engrailed 2 (EN2) is specifically involved in patterning the region that gives rise to the cerebellum and controls the plasticity of midbrain dopaminergic neurons. We carried out an association study for a CA repeat polymorphism located in the 3' region of the homeogene EN2. The subjects consisted of 165 patients with...
schizophrenia and 97 controls matched for age and ethnicity from a French Caucasian population. We found no significant association of schizophrenia with this bi-nucleotide repeat polymorphism of the EN2 gene.


http://www.sciencedirect.com/science/article/B6TBV-45DVXT1-1/2/7d871ec0aa9fb4172ca29136fe8914a3

We tested whether the human Clock (hClock) gene, one of the essential components of the circadian oscillator, is implicated in the vulnerability to delayed sleep phase syndrome (DSPS) and non-24-hour sleep-wake syndrome (N-24). Screening in the entire coding region of the hClock gene with PCR amplification revealed three polymorphisms, of which two predicted the amino acid substitutions R533Q and H542R. The frequencies of the R533Q and H542R alleles in patients with DSPS or N-24 were very low and not significantly different from those in control subjects. A T3111C polymorphism in the 3'-untranslated region of hClock, which had been reportedly associated with morning or evening preference for activity, was also investigated; the results showed that the 3111C allele frequency decreased in DSPS. Polymorphisms in the coding region of the hClock gene are unlikely to play an important role in the development of DSPS or N-24. The possible contribution of the T3111C polymorphism to DSPS susceptibility should be studied further.


http://www.sciencedirect.com/science/article/B6TBV-46041G4-9F/2/c62461c9863714131a986cf233a5c416

Association studies offer a promising tool to investigate the potential role of DNA sequence variation affecting the expression or sequence of proteins in susceptibility to common diseases. We determined the frequency of a DNA polymorphism resulting in a glycine to serine substitution at position 9 in the extracellular N-terminal part of the dopamine D3 receptor protein in a sample of 83 patients suffering from bipolar affective disorder and 100 control subjects. No significant differences between the groups were found. Thus, this substitution, which is the first sequence variation identified in the dopamine D3 receptor gene altering the amino acid sequence of the protein, can be regarded as a protein variant with no major effect on the susceptibility to bipolar affective disorder.

Pulmonary Pharmacology & Therapeutics  (1)

Congenital diaphragmatic hernia (CDH) is associated with high neonatal mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension. Antenatal interventions have been developed in an attempt to reduce the unacceptable mortality rate of CDH. The pathogenesis of pulmonary hypoplasia is not fully understood. It is not clear whether the increase of lung growth would be necessary for diaphragmatic closure. Vitamin A is important for various aspects of lung development. Therefore, the aim of this study was to examine whether antenatal treatment with vitamin A can increase lung growth and reduce the incidence of CDH in a nitrofen-treated rat model. The animals were randomly assigned to four groups: control, vitamin A, nitrofen, and nitrofen/vitamin A (NIP/Vit A). The incidence of CDH in the NIP/Vit A group (54%) was markedly lower than that in the nitrofen-treated group (85%). Although lung weight was decreased in the nitrofen-treated and NIP/vitamin A groups, the fetal lung weight-to-body weight ratio was slightly increased in the NIP/vitamin A group, compared to the nitrofen-treated group. The mRNA levels of lung surfactant proteins were decreased in the NIP/vitamin A group. We conclude that antenatal treatment with vitamin A reduced the incidence of CDH without lung maturation in the nitrofen-induced rat model.

Quaternary Research (1)


Advancements in ancient DNA analyses now permit comparative molecular and morphological studies of extinct animal dung commonly preserved in caves of semiarid regions. These new techniques are showcased using a unique dung deposit preserved in a late glacial vizcacha (Lagidium sp.) midden from a limestone cave in southwestern Argentina (38.5[deg] S). Phylogenetic analyses of the mitochondrial DNA show that the dung originated from a small ground sloth species not yet represented by skeletal material in the region, and not closely related to any of the four previously sequenced extinct and extant sloth species. Analyses of pollen and plant cuticles, as well as analyses of the chloroplast DNA, show that the Cuchillo Cura ground sloth browsed on many of the same herb, grass, and shrub genera common at the site today, and that its habitat was treeless Patagonian scrub-steppe. We envision a day when molecular analyses are used routinely to supplement morphological identifications and possibly to provide a time-lapse view of molecular diversification.

Radiotherapy and Oncology (6)

Alsner, J., S. B. Sorensen, et al. (2001). "TP53 mutation is related to poor prognosis after radiotherapy,
Background and Purpose: TP53 gene-mutation and expression of p53 have been described to influence the radiosensitivity of tumour cells from head and neck carcinomas. The present study was performed to evaluate whether TP53 mutation may influence the clinical outcome of head and neck cancer patients treated with radiotherapy or surgery.

Materials and methods: DNA was extracted from formalin-fixed paraffin-embedded tissue sections from primary biopsies taken before radiotherapy. Gene mutations (in exons 5-9) were identified using denaturing gradient gel electrophoresis (DGGE) as the initial scanning procedure and characterized by sequencing. Patients were treated with primary radiotherapy or surgery alone. Treatment was given according to the DAHANCA schedules with 5 or 6 weekly fractions (2 Gy) of radiotherapy (66-68 Gy). Most patients were also treated with the hypoxic radiosensitizer Nimorazole. The results are reported as 5-year actuarial values, and differences estimated by log-rank analysis.

Results: The present analysis is based on 114 patients with squamous cell carcinoma of the larynx, pharynx and oral cavity diagnosed between March 1992 and October 1996. Ninety patients received primary radiotherapy alone and 24 were treated with surgery. TP53 mutations were found in 45 patients (39%) and in patients receiving radiotherapy, TP53 mutation was highly associated with poor prognosis. Loco-regional control rates (5-year actuarial values) for TP53 mutation was 29 vs. 54% for TP53 wildtype (PPTP53 mutation did not influence the response to surgery.)

Conclusions: A strong relationship was observed between TP53 mutation and poor prognosis (increased risk of loco-regional failure and death) in head and neck cancer patients given primary radiotherapy but not surgery.


Recent studies suggest that normal tissue radiosensitivity is influenced by single nucleotide polymorphisms (SNPs) in certain genes. In order to seek a confirmation of these findings, this study investigated SNPs in genes TGFβ1 (position -509, codon 10 and codon 25), SOD2 (codon 16), XRCC1 (codon 399), XRCC3 (codon 241), APEX (codon 148) and ATM (codon 1853) in 26 breast cancer patients with marked changes in breast appearance after radiotherapy and 26 matched controls. Statistically significant associations were found between the TGFβ1 codon 10 Pro allele (P=0.005) as well as the TGFβ1 position -509 T allele (P=0.018) and increased risk of altered breast appearance. No significant associations were found for the remaining SNPs.


http://www.sciencedirect.com/science/article/B6TBY-4B3FY8Y-3/2/e46e6c8f00a759380c2c733bd03550ff5

Background and purpose: Single nucleotide polymorphisms (SNPs) in genes related to the biological response to radiation injury may affect clinical normal tissue radiosensitivity. This study investigates whether seven selected SNPs in five candidate genes influence risk of subcutaneous fibrosis and telangiectasia after radiotherapy. Patients and methods: The 41 patients included in
this study were given post-mastectomy radiotherapy in 1978-1982 and subsequently evaluated in detail with regard to several different normal tissue reactions. SNPs in TGFB1 (codons 10, 25 and position -509), SOD2 (codon 16), XRCC3 (codon 241), XRCC1 (codon 399) and APEX (codon 148) were analyzed by PCR and single nucleotide primer extension. Dose-response curves were established for subcutaneous fibrosis and telangiectasia in patients with different genotypes. Differences in radiosensitivity were quantified in terms of ED50 values and enhancement ratios.

Results For TGFB1, the Pro/Pro genotype in codon 10 and the T/T genotype in position -509 correlated positively with risk of subcutaneous fibrosis. The SOD 2 codon 16 Val/Ala genotype was associated with increased risk of subcutaneous fibrosis when compared to the Val/Val genotype. The Thr/Thr genotype in XRCC3 codon 241 correlated with increased risk of subcutaneous fibrosis as well as telangiectasia. The Arg/Arg genotype in XRCC1 codon 399 was associated with increased risk of radiation-induced subcutaneous fibrosis. For these polymorphisms, enhancement ratios between 1.09 and 1.25 were found. Combined analysis of multiple SNPs demonstrated that the risk of subcutaneous fibrosis correlated with the number of risk alleles in such a manner that patients with few risk alleles exhibited a remarkable degree of radioresistance.

Conclusion The present study established significant correlations between five SNPs and risk of radiation-induced normal tissue reactions. These findings support the assumption that clinical normal tissue radiosensitivity should be regarded as a phenomenon dependent on the combined effect of variation in several genes and indicate that models based on multiple genetic markers may have the potential to predict normal tissue responses after radiotherapy.


http://www.sciencedirect.com/science/article/B6TBY-4D1DKN6-1/2/b7648e36f23129015a9bff45d8d24ab7

Background and purpose An increasing amount of evidence indicates that single nucleotide polymorphisms (SNPs) may affect a variety of oncology related phenotypes. Occasionally, it is convenient to base studies addressing genotype-phenotype relationships on historical patient cohorts, from which only archival specimens are available. This study was conducted to validate protocols optimised for assessment of SNPs based on paraffin embedded, formalin fixed tissue samples.

Patients and methods In 137 breast cancer patients, three TGFB1 SNPs were assessed based on archival histological specimens. In 37 of these patients, the SNPs were also assessed using cultured fibroblasts and the assays were validated by direct comparison of the results. From the remaining 100 patients, only archival material was available. In these patients, the existence of a genetic linkage pattern between the assessed TGFB1 SNPs was used to provide an indirect validation of the genotyping results. Furthermore, two different methods for DNA extraction were compared (semi-automatic DNA extraction using the ABI Prism[trademark] 6100 Nucleic Acid PrepStation versus Proteinase K digestion for 5 days followed by boiling and DNA precipitation).

Results Assessment of SNPs based on archival histological material is encumbered by a number of obstacles and pitfalls. However, these can be widely overcome by careful optimisation of the methods used for sample selection, DNA extraction and PCR. Within 130 samples that fulfil the criteria for analysis a highly reliable SNP assessment was observed. The study demonstrated that different ‘down-stream applications’ (‘single nucleotide primer extension’ or ‘TaqMan[trademark]-based’ real-time PCR) could be used as genotyping procedure.

Conclusions Reliable assessment of SNPs in formalin-fixed paraffin-embedded specimens is possible but a number of precautions should be carefully taken.

Background and purpose: Preclinical evidence suggesting gemcitabine potentiates the anti-tumor effects of irradiation has resulted in clinical trials to evaluate the treatment efficacy of gemcitabine and concurrent thoracic irradiation in non-small-cell lung cancer (NSCLC). Although these studies demonstrated favorable tumor response, this combined treatment modality was accompanied by severe treatment-related toxicities predominantly of the lung. In an attempt to elucidate the determinants of lung toxicity for gemcitabine, we analyzed the expression of the pro-inflammatory cytokines TNF-[alpha], IL-1[alpha] and IL-6 in the lung tissue of mice treated with gemcitabine and concurrent thoracic irradiation.

Materials and methods: Four study groups were defined: C57BL/6J mice that received neither irradiation nor gemcitabine (NT-group), those that received gemcitabine (120 mg/kg intraperitoneal, i.p.) but no irradiation (GEM-group), those that underwent thoracic irradiation (12 Gy) without gemcitabine (XRT-group), and those that received both gemcitabine (120 mg/kg i.p., 2 h before irradiation) and thoracic irradiation (GEM/XRT-group). The mice were sacrificed at 1 h, 1 and 3 days, 1, 2 and 4 weeks post-treatment (p.t.). The mRNA expression of TNF-[alpha], IL-1[alpha] and IL-6 in the lung tissue was quantified by competitive RT-PCR. The cellular origin of the cytokine expression was identified by immunohistochemistry. The cytokine expression was correlated with histopathological alterations.

Results: The TNF-[alpha], IL-1[alpha] and IL-6 expression in the lung tissue of the GEM/XRT mice was clearly higher at all assessment time points compared to the NT mice (statistically significant at 1 h, 1 and 3 days, 1, 2 and 4 weeks p.t.), XRT mice (statistically significant at 1 week p.t.) or GEM mice (statistically significant at 1 h, 1 and 2 weeks p.t.). Maximal treatment-induced cytokine expression in the lung tissue of the GEM/XRT mice occurred already at 1 week p.t. (TNF-[alpha]: 30.9+/-5.3/IL-1[alpha]: 28.3+/-5.0/IL-6: 4.9+/-0.1 times basal level), and coincides with pathohistologically discernable interstitial pneumonitis. The elevated levels of TNF-[alpha] and IL-1[alpha] have been found to correlate with immunohistochemical staining of the bronchiolar epithelium and predominantly of inflammatory cells.

Conclusions: Our data provide evidence that the increased expression of pro-inflammatory cytokines and the induction of a cytokine-triggered inflammatory response may be a determinant of the observed elevated lung toxicity after concurrent treatment with gemcitabine and thoracic irradiation.


Purpose: The lung is the major dose-limiting organ for radiotherapy of cancer in the thoracic region. Immediate cellular damage after irradiation is supposed to result in cytokine-mediated multicellular interactions with induction and progression of inflammatory and fibrotic tissue reactions. Pentoxifylline (PTX) down-regulates the production of proinflammatory cytokines, particularly TNF-[alpha], in response to noxious stimuli and may therefore provide protection against radiation-induced, cytokine-mediated cellular damage. The purpose of this study was to investigate the temporal and spatial release of TNF-[alpha] in the lung tissue after thoracic irradiation with 12 Gy. In addition, we evaluated the ability of PTX to reduce the radiation-induced TNF-[alpha] release in this animal model of thoracic irradiation.

Materials and methods: C57BL/6J mice were exposed to either sham irradiation or single fraction of 12 Gy delivered to the thorax. Four study groups were defined: those that received neither irradiation nor PTX (NT group), those that received PTX but no irradiation (PTX group), those that underwent irradiation without PTX.
(XRT group) and those that received both PTX and irradiation (PTX/XRT group). Treated and sham-irradiated mice were sacrificed corresponding to the latent period and the pneumonic phase. The TNF-[alpha] mRNA expression in the lung tissue was quantified by 'real-time' quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Immunohistochemical detection methods (alkaline phosphatase anti-alkaline phosphatase (APAAP)) and automated image analysis were used for objective quantification of TNF-[alpha] protein expression. Results: Following thoracic irradiation with a single dose of 12 Gy (XRT group), radiation-induced TNF-[alpha] mRNA release in the lung tissue was significantly increased during the acute phase of pneumonitis (PP).

Conclusions: This study demonstrates a significant radiation-induced increase of TNF-[alpha] (on mRNA and protein level) in the lung tissue during the pneumonic phase. The predominant localisation of TNF-[alpha] in areas of inflammatory cell infiltrates suggests involvement of this cytokine in the pathogenesis of radiation-induced lung injury. In addition, we observed a pronounced reduction of the TNF-[alpha] mRNA and protein production in the study group that received both PTX and radiation (PTX/XRT group) as compared to the radiation-only group (XRT group). Therefore our results indicate that PTX down-regulates the TNF-[alpha] mRNA and protein production in the lung tissue in response to radiation.

Recent Prog. Horm. Res. (1)


http://rphr.endojournals.org/cgi/content/abstract/58/1/155

We have undertaken a high-throughput analysis to identify targets of glucocorticoid regulation in P1798 murine T-lymphoma cells. G1/S-arrested cultures were treated for 8 hours with 0.1 {micro}M dexamethasone (dex) in the presence and absence of 1 {micro}g/ml cycloheximide. Untreated cultures and cultures exposed to cycloheximide alone were prepared as controls. RNA was isolated and gene expression analyzed using Affymetrix MG-U74A oligonucleotide arrays (Gene Chips(R)). Three independent experiments were performed. The data were analyzed using a variety of statistical and analytical approaches in order to identify primary transcriptional targets of the glucocorticoid receptor. We identified 44 genes that increase by > 2-fold in both dex-treated and dex + cycloheximide-treated cultures (relative to control and cycloheximide-treated cultures) in three replicate experiments. Statistical analysis of control data indicate that the probability that a given probeset would, as a result of random error, increase > 2-fold both in the presence and absence of cycloheximide in two independent experiments is approximately 7 x 10-9. We have retrieved from the Celera mouse genomic sequence 8 kb of promoter sequence, spanning 4 kb either side of the 5'-end of the cDNA from eight of the induced genes. These sequences were analyzed for potential glucocorticoid receptor binding sites. Five of these genes contain the sequence ACAnnnTGTnCT within 4 kb of the presumptive transcriptional start site. Eight control genes were selected at random and analyzed for the sequence ACAnnnTGTnCT. Two control genes had such sequences within 4 kb of the transcriptional start site.

Regulatory Peptides (24)

http://www.sciencedirect.com/science/article/B6T0S-3YRW08S-9/2/5790e44054927ad9ad53096e723209ae

Somatostatin receptor expression is a favorable prognostic factor in human neuroblastoma. Somatostatin receptors have been demonstrated in vitro by pharmacologic analysis of tumor tissue and in vivo by diagnostic radioreceptor scintigraphy. However, which receptor subtypes (sst1, sst2, sst3, sst4, and sst5) are expressed in these tumors has not yet been delineated. We used RT-PCR to analyze expression of the five somatostatin receptor genes in 32 neuroblastoma tumor specimens. All 32 tumor specimens expressed mRNA for c-abl and sst1; sst2 mRNA was detected in 27/32 samples and somatostatin mRNA was detected in 30/32 tumor specimens. The remaining receptor subtypes, sst3, sst4, and sst5 were variably expressed. Receptor protein for sst1 and sst2 was visualized in tumor neuroblasts as well as in endothelial cells of tumor vessels using immunostaining with specific anti-receptor antibodies. The effect of high expression of somatostatin receptors on cell proliferation was examined in SKNSH neuroblastoma cells transfected with sst1 and sst2. SS14 binding to wild-type SKNSH cells was undetectable; but the native peptide bound with high affinity to the SKNSH/sst1 and SKNSH/sst2 neuroblastoma cell lines. Pharmacologic analysis of binding with two long-acting analogues, CH275 and octreotide, confirmed selective expression of sst1 and sst2 in stably transfected SKNSH cells. Formation of neuroblastoma xenograft tumors in nude mice was significantly delayed for both SKNSH/sst1 (P<0.01 and sst2, are expressed in the majority of neuroblastomas at diagnosis; and (2) upregulation of functional sst1 or sst2 in neuroblastoma cell lines suppresses tumorigenicity in a xenograft model. These observations suggest that somatostatin receptors may be a useful therapeutic target in neuroblastoma.


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We hypothesize that vasoactive intestinal peptide (VIP) promotes neural crest differentiation through VIP receptor type I (VPAC1). In order to test this hypothesis, SKNSH neuroblastoma cells were stably transfected with VPAC1 and receptor expression was verified by real-time RT-PCR. Overexpression of VPAC1 in SKNSH cells resulted in upregulation of endogenous retinoic acid receptor expression for both RAR[alpha] and RXR[alpha] with no change in expression of RAR[beta]. Transfected cells demonstrated high affinity binding of VIP (KD=0.2 nM) and VIP-mediated stimulation of adenylate cyclase and a shift in cell cycle kinetics to a near triploid DNA index in G1. SKNSH/VPAC1 cells treated with VIP were observed to express a more differentiated phenotype compared to wild type cells as characterized by an increase in tissue transglutaminase II and a decrease in bcl-2 immunostaining. VIP-induced differentiation effects were potentiated by retinoic acid. This differentiation resulted in decreased proliferative potential in a xenograft model. Whereas, wild type SKNSH cells induced tumor growth in 100% of nude mice within 13 days post-injection, SKNSH transfected with VPAC1 demonstrated no tumor formation in xenografts followed for 6 months. Taken together, these data support the hypothesis that VIP modulation of neural crest differentiation is mediated via VPAC1 and that high expression of VPAC1 induces differentiation in and decreases tumorigenicity of neuroblastoma cells.
The histamine-producing ECL cells are numerous in the acid-producing (oxyntic) mucosa. They respond to gastrin by secretion of histamine that acts on parietal cells to produce acid. In addition, gastrin has a trophic effect on the oxyntic mucosa which is exerted on stem cells and ECL cells. To elucidate the molecular actions of gastrin on the stomach we attempted to identify genes that are regulated by gastrin in oxyntic mucosa and in isolated ECL cells. Differential display polymerase chain reaction was used to identify mRNAs that are differentially expressed in rats that are hypergastrinemic after treatment with the proton pump inhibitor omeprazole for 48 h compared with rats that are hypogastrinemic after 24 h fasting. Differences in mRNA levels were confirmed by Northern blot analysis (comparing mRNA from fasted rats, omeprazole-treated rats and rats treated with omeprazole+the CCK2 (cholecystokinin) receptor antagonist YF476). The cDNAs were identified by sequencing followed by database search. Hypergastrinemia induced by omeprazole treatment resulted in overexpression of mRNA for histidine decarboxylase, fetuin, pepsinogen and cytochrome P450 in the oxyntic mucosa. This was prevented by CCK2 receptor blockade. In isolated ECL cells gastrin upregulated mRNAs for histidine decarboxylase and synaptotagmin V as well as one mRNA transcript without known homology.

NPY is a 36-amino acid peptide which exerts its physiological effects through the activation of a family of G-protein coupled receptors. In vivo and in vitro characterization of the recently cloned rat Y5 receptor suggests that it is a primary mediator of NPY-induced feeding (Gerald et al., Nature 1996;382:168-171). We now report the molecular cloning and pharmacological characterization of the human, dog and mouse homologs of the Y5 receptor. With the exception of a 21 amino acid repeat in the amino terminus of the mouse Y5 receptor, the sequence of the four species homologs appear to be highly conserved, with 88% to 97% amino acid identities between any two species. Similarly, the pharmacological profiles of the four species homologs as determined in porcine 125I-PYY binding assays show a great deal of conservation, with the following rank order of affinity: human or porcine NPY, PYY, [Leu31,Pro34]NPY, NPY2-36, human PP>human [-Trp32]NPY>rat PP, C2-NPY. Northern blot analysis reveals that the Y5 receptor is widely distributed in the human brain, with the strongest signals detected in the cortex, putamen and caudate nucleus. The chromosomal localization of the human Y5 receptor, previously shown to be overlapping and in the opposite orientation to the Y1 receptor, is determined to be 4q31, the same locus as previously demonstrated for the human Y1 receptor (Herzog et al., J Biol Chem 1993;268:6703-6707), suggesting that these receptors may be coregulated. These Y5 species homologs along with corresponding animal models may be useful in the search for novel therapeutics in the treatment of obesity and related feeding disorders.
Splice variants (SV) of receptors for growth hormone-releasing hormone (GHRH) have been found in several human cancer cell lines. GHRH antagonists inhibit growth of various human cancers, including osteosarcomas and Ewing's sarcoma, xenografted into nude mice or cultured in vitro and their antiproliferative action could be mediated, in part, through these SV of GHRH receptors. In this study, we found mRNA for the SV1 isoform of GHRH receptors in human osteosarcoma line MNNG/HOS and SK-ES-1 Ewing's sarcoma line. We also detected mRNA for GHRH, which is apparently translated into the GHRH peptide and secreted by the cells, as shown by the presence of GHRH-like immunoreactivity in the conditioned media of cell cultures. In proliferation studies in vitro, the growth of SK-ES-1 and MNNG/HOS cells was dose-dependently inhibited by GHRH antagonist JV-1-38 and an antiserum against human GHRH. Our study indicates the presence of an autocrine stimulatory loop based on GHRH and SV1 of GHRH receptors in human sarcomas. The direct antiproliferative effects of GHRH antagonists on malignant bone tumors appear to be exerted through the SV1 of GHRH receptors on tumoral cells.


Intestinal trefoil factor (TFF3) is essential in regulating cell migration and maintaining mucosal integrity in gastrointestinal tract. We previously showed that TFF3 was overexpressed in gastric carcinoma. Whether TFF3 possesses malignant potential is not fully elucidated. We sought to investigate the effects of inducing TFF3 expression in a non-malignant rat fibroblast cell line (Rat-2) on the cell proliferation, invasion and the genes regulating cell invasion. Invasiveness and proliferation of transfected Rat-2 cell line were assessed using in vitro invasion chamber assay and colorimetric MTS assay. Differential mRNA expressions of invasion-related genes, namely, metalloproteinases (MMP-9), tissue inhibitors of metalloproteinases (TIMP-1), [beta]-catenin and E-cadherin, were determined by quantitative real-time polymerase chain reaction (PCR). We showed that TFF3 did not inhibit the proliferation of Rat-2 cells. We also demonstrated that transfection of TFF3 significantly promoted invasion of Rat-2 cells by 1.4- to 2.2-folds. There was an upregulation of [beta]-catenin (13.1-23.0%) and MMP-9 (43.4-92.2%) mRNA expression levels, and downregulation of E-cadherin (25.6-33.8%) and TIMP-1 (31.5-37.8%) in TFF3-transfected cells compared to controls during 48-h incubation. Our results suggested that TFF3 possesses malignant potential through promotion of cell invasiveness and alteration of invasion-related genes.


Vasoactive intestinal peptide (VIP) upregulates the expression of vascular endothelial cell growth
factor (VEGF189, VEGF165 and VEGF121) mRNAs in human prostate cancer LNCaP cells, as shown by reverse transcriptase-polymerase chain reaction (RT-PCR). Real-time RT-PCR indicated that the effect was maximal by 1-2 h and must be accounted for increased transcription since VIP decreased VEGF165 mRNA stability. VIP stimulated VEGF165 protein synthesis as measured by ELISA. VIP regulation of VEGF expression was mediated by VPAC1 receptor and was cAMP/protein kinase A (PKA) dependent. Phosphoinositide 3-kinase (PI3-K) and mitogen-activated protein kinase MEK1/2 systems may also be involved as shown with specific kinase inhibitors. These actions together with the observation of VIP-induced neuroendocrine differentiation in LNCaP cells suggest a proangiogenic potential of VIP in prostate cancer.


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Pituitary adenylate cyclase activating polypeptides (PACAP) and PAC1 receptor signaling have diverse roles in central and peripheral nervous system development and function. In recent microarray analyses for PACAP and PAC1 receptor modulation of neuronal transcripts, the mRNA of Homer 1a (H1a), which encodes the noncrosslinking and immediate early gene product isoform of Homer, was identified to be strongly upregulated in superior cervical ganglion (SCG) sympathetic neurons. Given the prominent roles of Homer in synaptogenesis, synaptic protein complex assembly and receptor/channel signaling, we have examined the ability for PACAP to induce H1a expression in sympathetic, cortical and hippocampal neurons to evaluate more comprehensively the roles of PACAP in synaptic function. In both central and peripheral neuronal cultures, PACAP peptides increased transiently H1a transcript levels approximately 3.5- to 6-fold. From real-time quantitative PCR measurements, the temporal patterns of PACAP-mediated H1a mRNA induction among the different neuronal cultures appeared similar although the onset of sympathetic H1a transcript expression appeared protracted. The increase in H1a transcripts was accompanied by increases in H1a protein levels. Comparative studies with VIP and PACAP(6-38) antagonist demonstrated that the PACAP effects reflected PAC1 receptor activation and signaling. The PAC1 receptor isoforms expressed in central and peripheral neurons can engage diverse intracellular second messenger systems, and studies using selective signaling pathway inhibitors demonstrated that the cyclic AMP/PKA and MEK/ERK cascades are principal mediators of the PACAP-mediated H1a induction response. In modulating H1a transcript and protein expression, these studies may implicate broad roles for PACAP and PAC1 receptor signaling in synaptic development and plasticity.


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The regulated expression of the peptide and transcript levels of the neurotrophic peptides, pituitary adenylate cyclase-activating polypeptide (PACAP), galanin and vasoactive intestinal peptide (VIP) were examined in sympathetic neurons of the rat superior cervical ganglion (SCG). Real-time quantitative PCR methods were developed to assess modulation of neuronal peptide precursor protein transcript levels following experimental paradigms of neuropeptidergic plasticity. Oligonucleotide primer, fluorogenic probe and amplification conditions were optimized for
maximal assay sensitivity. Depolarization of primary cultured sympathetic neurons stimulated PACAP, galanin, and VIP peptide contents and releases with differing magnitudes and temporal profiles. The rank order of increased neuronal peptide content paralleled the augmented peptide release (VIP>galanin>PACAP). Maximal cellular PACAP and VIP levels were achieved by 72 and 96 h, respectively; galanin levels did not plateau during the treatment period. PACAP transcript elevation was rapid and transient; PACAP mRNA expression diminished at longer depolarization times, which diverged markedly from the sustained high peptide production levels. By contrast, VIP and galanin mRNAs reached maximal levels at later times, and appeared to correlate more closely with peptide production. We previously described multiple proPACAP mRNA variants resulting from alternative 3’ untranslated region cleavage and polyadenylation. The shorter depolarization-induced PACAP transcripts exhibit longer half-lives, suggesting that the short proPACAP mRNA variant may function to impart PACAP translational efficiency and sustain PACAP peptide production.


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Vasoactive intestinal peptide (VIP) is a neurotransmitter with neurotropic effects. VIP functions through two distinct G-protein-coupled receptor subtypes (VPAC1 and VPAC2). We have demonstrated expression of VPAC1 in pediatric nervous system tumors, including medulloblastoma arising in the cerebellum and neuroblastoma arising in the adrenal medulla. More recently, we have reported the differentiation of neuroblastoma cells by upregulation of VIP type 1 receptor suggesting a role for VPAC1 in neuronal development. To understand the molecular mechanisms regulating VPAC1 expression in both cerebellum and adrenal medulla, we have cloned the human VPAC1 gene and sequenced 2.6-kb of the 5’-flanking sequence. Expression of the luciferase reporter gene under the control of this 2.6-kb human VPAC1 promoter was induced 35-fold in a human medulloblastoma cell line (DAOY) and 36-fold in a human neuroblastoma cell line (SKNSH). Analysis of 5'-unidirectional deletion derivatives of the 2.6-kb fragment demonstrated that a 241-bp sequence immediately upstream of the VPAC1 coding region retains high activity, suggesting that it contains the core promoter region. Quantitative RT-PCR analysis demonstrated that VPAC1 is expressed in mouse cerebellar and adrenal tissues. The VPAC1 promoter also directed expression of a reporter gene in cerebellum and adrenal medulla in transgenic mice. Along with our previous findings, these results suggest that VPAC1 may play a functional role in development of both cerebellum and adrenal medulla.

The three subtypes of peroxisome proliferator activated receptors (PPAR[alpha], [delta] and [gamma]) control the storage and metabolism of fatty acids. Treatment of rats with the PPAR[alpha] ligand ciprofibrate increases serum gastrin concentrations, and several lines of evidence suggest that non-amidated gastrins act as growth factors for the colonic mucosa. The aim of the present study was to investigate the expression of PPARs and the effect of PPAR ligands on gastrin production and cell proliferation in human colorectal carcinoma (CRC) cell lines. mRNAs for all three PPAR subtypes were detected by PCR in all CRC cell lines tested. The concentrations of progastrin, but not of glycine-extended or amidated gastrin, measured by radioimmunoassay in LIM 1899 conditioned media and cell extracts were significantly increased by treatment with the PPAR[alpha] ligand clofibrate. Similar increases in progastrin were seen following treatment with the PPAR[alpha] ligands ciprofibrate and fenofibrate, but not with bezafibrate, gemfibrozil or Wy 14643. The PPAR[gamma] agonist rosiglitazone had no significant effect on progastrin production. The PPAR[alpha] ligand clofibrate also stimulated proliferation of the LIM 1899 cell line. We conclude that some PPAR[alpha] ligands increase progastrin production by the human CRC cell line LIM 1899, and that clofibrate increases proliferation of LIM 1899 cells. These studies have revealed a relationship between PPARs and gastrin, two regulatory molecules implicated in the pathogenesis of CRC.


Cloning with subsequent in vitro and in vivo characterization of vascular neuropeptide Y (NPY) receptor subtypes in porcine and canine peripheral tissues was performed. RT-PCR with Y1 and Y2 receptor-specific primers, indicated expression of Y1 receptors in both kidney and spleen of dog and pig, and expression of Y2 receptors in pig spleen. In pig kidney, expression of Y1 receptor mRNA was located to intrarenal arteries, as demonstrated with in situ hybridization using human probes. The cloned and sequenced canine Y1, porcine Y1 and Y2 receptors revealed high homologies to previously characterized mammalian NPY receptors. Membrane and autoradiographic receptor-binding studies showed specific high-affinity binding sites for the purported Y1-selective radioligands 125I-[Leu31Pro34]peptide YY (PYY) and 3H-BIBP 3226 in dog spleen, and for the putative Y2-selective 125I-PYY(3-36) in dog and pig spleen. In the pig in vivo, [Leu31Pro34]PYY, administered i.v., evoked vasoconstriction in spleen and kidney, actions that were potently inhibited by the non-peptide Y1 receptor antagonist SR 120107A. In contrast, PYY(3-36) evoked vasoconstriction only in spleen and this effect was not influenced by SR 120107A. NPY evoked renal and splenic vasoconstriction in the dog in vivo, vascular responses that were inhibited by both BIBP 3226 and SR 120107A. Furthermore, the Y1 receptor agonist [Leu31Pro34]NPY also caused vasoconstriction in dog kidney and spleen, whereas the putative Y2 agonist N-acetyl[Leu28Leu31]NPY(24-36) evoked no such vascular responses. It is concluded that the pig spleen is likely to contain Y1 and Y2 receptors, both involved in splenic vasoconstriction. In contrast, the Y1 receptor seems to be the sole vascular NPY receptor subtype in pig kidney. Moreover, Y1 receptors predominate in dog spleen and kidney. Furthermore, the cloned canine Y1 receptor and the porcine Y1 and Y2 receptors show great homologies to, and possess ligand requirement profiles in accordance with, the human forms.

Using reverse transcriptase-polymerase chain reaction, products corresponding to mRNA encoding endothelin-A and -B (ETA and ETB) receptors were demonstrated in human coronary arteries and veins with intact endothelium and in endothelium-denuded human coronary arteries. Vasomotor responses were studied on isolated segments of human epicardial coronary arteries and veins at resting tension and after precontraction with U46619. In both arteries and veins, endothelin-1 (ET) induced strong and potent contractions, and preincubation with different concentrations of the non-selective ETA/ETB receptor antagonist PD 145065 caused a rightward shift of the concentration-response curves without significantly changing maximum responses (pA2 value 6.7 arteries, 7.4 veins). The ETB receptor agonist IRL 1620 induced no contraction of arteries or veins at resting tension, but induced weak relaxation of all arteries and most precontracted veins, the relaxation being endothelium-dependent in arteries. ET at low concentrations induced weak relaxations of most precontracted arteries, but no veins. In conclusion, mRNA encoding ETA and ETB receptors is present in human coronary arteries and veins, ETA receptors mediating contraction and ETB receptors mediating relaxation. In arteries, mRNA for both receptor types was detected in the media, but ETB receptor-mediated relaxation was endothelium-dependent.


Rats exposed chronically to a cold environment (5 [deg]C/4 [deg]F) develop hypertension. This cold-induced hypertension (CIH) is a non-genetic, non-pharmacological, non-surgical model of environmentally induced hypertension in rats. The renin-angiotensin system (RAS) appears to play a role in both initiating and/or maintaining the high blood pressure in CIH. The goal of the present study was to evaluate the role of central and peripheral circulating RAS components, angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin (Ang) II, in CIH. Seventy-two Sprague-Dawley adult male rats were used. Thirty-six rats were kept in cold room at 5 [deg]C while the other 36 were at 24 [deg]C as controls for 5 weeks. Systolic blood pressure (SBP) was recorded by tail cuff. The SBP was increased in rats exposed to cold within 1 week, and this increase was significant for the next 2-5 weeks of the cold exposure (p<0.05, n=12) were sacrificed at 1, 3 and 5 weeks. The brain and liver were removed and plasma was saved. The AGT mRNA significantly increased in the hypothalamus and liver in cold-treated rats from the first week of exposure to cold, and was maintained throughout the time of exposure to cold (n=4, pp>0.05, n=4). The hypothalamic Ang II levels were significantly increased, whereas plasma Ang II levels significantly decreased, in the rats of 5 weeks of cold exposure (n=8, p<0.05, n=12). The results show differential regulation of RAS components, AGT, ACE and Ang II, between brain and periphery in cold-exposed rats. We conclude that the exposure to low temperature initially increases plasma RAS but with continuous exposure to cold, the brain RAS maintains the hypertension, probably by sustained sympathetic activation, which would provide increased metabolism but also vasoconstriction leading to hypertension.

Rats exposed chronically to mild cold (5[deg][C]/41[deg][F]) develop hypertension. This cold-induced hypertension (CIH) is an environmentally induced, non-surgical, non-pharmacological and non-genetic model for studying hypertension in rats. The blood renin angiotensin system (RAS) appears to play a role in both initiating and maintaining the high blood pressure in CIH. The goal of the present study was to evaluate the role of brain angiotensin type 1 and type 2 receptors (AT1 R and AT2 R) in CIH. Sprague-Dawley adult male rats were used. Thirty-six rats were kept in a cold room at 5[deg][C] and the other 36 were kept at 24[deg][C] as controls. Systolic blood pressure (SBP) was recorded by tail cuff. The SBP was elevated in rats exposed to cold within 1 week (n=12, P>0.05), significantly increased at 3 weeks (PP1 R and AT2 R. The AT1 R mRNA was increased significantly in hypothalamus and brainstem after the first week in cold-treated rats and was maintained throughout the time of exposure to cold (n=6, P1 R binding significantly increased initially in hypothalamus and thereafter in brainstem. The mRNA and the receptor binding for AT2 R decreased significantly (Pn=6) in nucleus of inferior olive and locus coeruleus of brainstem in cold-treated rats after exposure to cold. The experiments show differential regulation of RAS components, AT1 R and AT2 R, in different brain areas in cold-exposed rats and provide evidence that up-regulated AT1 R and down-regulated AT2 R in different brain areas are involved in CIH. The opposing directions of expression of AT1 R and AT2 R suggest that they play counterbalancing roles in brain function.


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We reported previously that the expression of type 2 somatostatin receptor (sst2) was positively related to patient outcome in the childhood tumor neuroblastoma. To quantitate the expression of mRNA sst2 expression, we used a competitive RT-PCR assay. To improve the practicability of this measurement and its applicability to large groups of patients, we present here an original 'real-time' quantitative RT-PCR method, based on a dual-labeled fluorogenic probe and the TaqMan(TM) technology. By this method, we have measured sst2 mRNA expression in 24 breast cancer samples and 26 colon carcinomas as well as on the corresponding non-adjacent non-neoplastic tissue of the same patients. The proposed method has a dynamic range of 4 x 104 to 4 x 108 molecules of sst2 mRNA. The intra-assay precision of the test, evaluated as signal detection variability, was 2.4%. Accuracy, evaluated by the addition of standard RNA to unknown samples, provided a mean recovery of 98+/−2%. A significant correlation has been observed in a study performed in 24 neuroblastoma samples measured both with the proposed method and with a competitive RT-PCR assay (r=0.913, p7+/−2.0 x 107 molecules/[mu]g total RNA, cancer tissue 9.7 x 107+/−4.2 x 107) and breast tumors (normal tissue 5.5 x 108+/−2.0 x 108, cancer tissue 4.4 x 108+/−3.7 x 108). However, in colorectal cancer, sst2 mRNA values of subjects with high circulating carcinoembryonic antigen (CEA) levels (>5 ng/ml) were statistically lower (2.3 x 107+/−6.2 x 106 molecules/[mu]g total RNA; p8+/−6.7 x 107). Also, the sst2 mRNA ratio between normal and tumor tissue (N/T ratio) resulted significantly inversely related to CEA levels. In breast cancer, a significant difference was found between the mean N/T ratio of negative (below 10 fmol/mg protein) and positive estrogen receptor tumors (pp<0.05). The proposed method is accurate, precise, sensitive and less labor-intensive than the competitive RT-PCR assay. For a correct evaluation of sst2 mRNA expression, it seems very important to measure the sst2 expression both in tumor and in the non-tumoral non-adjacent tumor specimens.

Five neuropeptide Y (NPY) receptor subtypes have been cloned in mammals. The degree of sequence conservation differs considerably between subtypes as well as between evolutionary lineages. To shed further light on this, we have cloned the five NPY receptors in the guinea pig. Here, we report the cloning of the guinea pig Y2 receptor. The Y2 receptor is generally highly conserved, with 90-95% identity between different orders of mammals, including the guinea pig. The guinea pig receptor has a divergent cytoplasmic tail, indicating possible differences in regulation of signalling and/or down regulation. COS-7 cells transiently transfected with the gpY2 receptor show saturable 125I-PYY binding with a Kd=6 pM. In displacement experiments, the gpY2 receptor was similar to the human and rat receptors with the following rank order of potencies: pNPY>pPYY>pNPY13-36=pNPY22-36>>[Leu31Pro34]NPY>BIBP3226. Thus, the guinea pig Y2 receptor is well conserved in comparison with human and rat with regard to both amino acid sequence and pharmacological profile.


Somatostatins are a diverse family of peptide hormones that regulate various aspects of growth, development, and metabolism through interactions with numerous somatostatin receptor subtypes (SSTRs) on target tissues. In this study, we used rainbow trout to evaluate the effects of growth hormone (GH), insulin (INS), and insulin-like growth factor-I (IGF-I) on the expression of SSTR 1A, 1B and 2 mRNAs. GH regulated the expression of SSTRs in a subtype- and tissue-specific manner. GH reduced SSTR 1A, 1B, and 2 expression in optic tectum, reduced SSTR 1A and 1B expression in pancreas, reduced hepatic SSTR 1B expression in liver, and increased hepatic SSTR 1B expression. INS also regulated SSTR expression in a subtype- and tissue-specific manner. INS reduced SSTR 1B expression in optic tectum, increased SSTR 2 expression in pancreas, and increased SSTR 1B and 2 expression in liver. IGF-I generally decreased the expression of all SSTRs. These data indicate that GH, INS, and IGF-I modulate the expression of SSTRs and suggest that independent mechanisms may serve to regulate the various receptor subtypes.


A common feature in asthma is the induction of reactive oxygen species (ROS) and the AP-1 transcription factor during the inflammatory process. AP-1 induction leads to an increased expression of pro-inflammatory cytokines. Also, higher levels of the pro-inflammatory neuropeptide substance P (SP) have been reported in bronchoalveolar-lavage fluid of asthmatics. Here, the role of SP on ROS induction and the downstream activation of AP-1 in A549 airway epithelial cells was investigated by dichlorofluorescein-diacetate method and reporter gene assays. The SP-mediated AP-1 induction was dependent on extracellular calcium and ROS. The likely source of ROS are the mitochondria as rotenone inhibited AP-1 induction and the...
p47phox subunit of the NADPH oxidase complex, responsible for ROS generation in phagocytic cells, was not expressed in A549 cells assayed by RT-PCR. This is consistent with results obtained from cells of murine bronchial epithelium, isolated by laser capture microdissection. In summary, this study provides evidence for an SP-mediated induction of AP-1, which may contribute to the expression of pro-inflammatory cytokines.


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It has been demonstrated that arginine vasopressin (AVP) is synthesized not only in specific hypothalamic nuclei, but also in the adrenal medulla where it is thought to regulate adrenal functions by autocrine and paracrine mechanisms. In order to further characterise the effects of AVP on rat adrenal chromaffin cells, we examined: (a) the mRNA expression for V1a and V1b AVP receptors in these cells; (b) the effects of AVP on the membrane potential and membrane currents measured with the whole-cell patch-clamp technique; and (c) effect of AVP on catecholamine release from single adrenal chromaffin cells measured with carbon fibre microelectrodes. Reverse transcription-polymerase chain reaction (RT-PCR) on tissue punch samples obtained from the adrenal medulla demonstrated message for both the V1a and V1b receptors, while material obtained from the adrenal cortex showed expression of the V1a receptor only. Single-cell RT-PCR conducted on acutely isolated chromaffin cells showed message for the V1a receptor in 84% of cells, while 38% of cells also contained message for the V1b receptor (n=45). Under current-clamp recording, responses to AVP application (4-40 [mu]M) were variable; 22/34 (65%) tested cells were depolarised, 29% hyperpolarised, and the remaining cells showed a biphasic response. Changes in membrane potential of either direction were dose-dependent and accompanied by a decrease in cell membrane resistance. Under voltage-clamp (Vhold=-60 mV), AVP evoked inward current in 27/52 (52%) and outward current in 16/52 (31%) chromaffin cells. Both types of AVP-evoked responses were blocked by co-application of a nonselective V1a/V1b antagonist. Application of AVP evoked prolonged bursts of amperometric currents (indicative of catecholamine release) in 4/9 tested cells, but reduced the currents evoked by ACh application in all tested cells (n=7). These findings demonstrate a complex action of AVP on adrenal chromaffin cells, with individual adrenal chromaffin cells responding with either excitation or inhibition. This response pattern may be related to the expression of V1 receptor subtypes.


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In both functional and radioligand binding studies of gastric smooth muscle from rabbit and guinea pig, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) show equal potency indicating that the receptor type is either a VIP1/PACAP2 or a VIP2/PACAP3 receptor. We have characterized the VIP/PACAP receptor expressed in freshly dispersed and cultured gastric and tenia coli smooth muscle cells of rabbit and guinea pig by reverse transcriptase-polymerase chain reaction (RT-PCR), Northern analysis, and cloning of the first extracellular domain. Specific primers based on cDNA sequences for rat VIP1/PACAP2, VIP2/PACAP3 and PACAP1 receptors were designed spanning the first extracellular domain. A 275 base pair product corresponding to VIP2/PACAP3 receptor was
amplified by RT-PCR in muscle cells from both species. No RT-PCR product was obtained with primers for VIP1/PACAP2 and PACAP1 receptors. The deduced amino acid sequences showed 90% similarity in rabbit and 77% in guinea pig to the sequence in rat. The location of the aspartate, tryptophan and glycine residues and all six N-terminal cysteines required for VIP binding were conserved. The sequence in guinea pig tenia coli differed from that in guinea pig stomach by two amino acid residues, Phe40 and Phe41. Northern analysis revealed a single 3.9 kilobase (kb) mRNA corresponding to VIP2/PACAP3 receptors in rabbit and a 2.1 kb mRNA in guinea pig gastric and tenia coli muscle cells. We conclude that only VIP2/PACAP3 receptors are expressed in smooth muscle cells of rabbit and guinea pig. The two amino acid difference in the sequence obtained from guinea pig tenia coli may reflect the distinct binding and functional properties of this tissue.


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Ghrelin was isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) and has been found in the gastrointestinal tract of many vertebrates. Although the sequence and structure of chicken ghrelin has recently been determined, morphological characteristics of ghrelin cells in the chicken gastrointestinal tract are still obscure. In this study, we investigated ghrelin expression and distribution of ghrelin-producing cells in the hatching and adult chicken gastrointestinal tract by RT-PCR, immunohistochemistry and in situ hybridization. Ghrelin mRNA expression was observed mainly in the proventriculus in the hatching chicken and in the proventriculus, pylorus and duodenum of the adult chicken by RT-PCR. Ghrelin-immunopositive (ghrelin-ip) cells in the proventriculus were located at the mucosal layer but not in the myenteric plexus or smooth muscle layer. The number of ghrelin-ip cells in the adult chicken was greater than that in the hatching chicken. Interestingly, in the adult chicken, the number of ghrelin-ip cells were almost the same as that of ghrelin mRNA-expressing (ghrelin-ex) cells; however, in the hatching chicken, the number of ghrelin-ex cells was greater than that of ghrelin-ip cells. These results clearly demonstrate that ghrelin-producing cells exist in the chicken gastrointestinal tract, especially in the proventriculus, from hatching to adult stages of development, as well as in mammals.


http://www.sciencedirect.com/science/article/B6T0S-4B5044P-4/2/c9ef8e3d82308cc49198ba16c5afe33

Previous studies have indicated a relationship between cocaine- and amphetamine-related transcript (CART) and leptin. The present study used quantitative PCR and in situ hybridization to examine this CART-leptin relationship in different animal models. With CART injection, the function of this pathway was also investigated. The results demonstrate that CART mRNA in the arcuate nucleus (ARC) was significantly increased in subjects fed a high-fat diet (HFD) compared to low-fat diet (LFD). It was also elevated in obese vs. lean rats and in normal-weight obesity-prone vs. obesity-resistant rats. In each group tested, CART mRNA in the ARC was positively correlated specifically with circulating levels of leptin. Its close association specifically with leptin was further supported by a stimulatory effect of this hormone on CART expression. This leptin-CART relationship in the ARC, in contrast, was less consistent or undetectable in the
paraventricular nucleus and lateral hypothalamus. Central injection of CART peptide (55-102) increased circulating non-esterified fatty acid levels and decreased lipoprotein lipase activity in adipose tissue. These results suggest that, on a fat-rich diet, this leptin-CART pathway originating in the ARC inhibits excessive body fat accrual by causing a shift from lipid storage toward lipid mobilization.

Reproduction (4)


http://www.reproduction-online.org/cgi/content/abstract/124/3/387

Expression and activity of the Na-K-ATPase within the basolateral membrane domains of the trophectoderm epithelium provide the driving force for accumulation of Na(+) and Cl(-) across the nascent epithelium, mediating fluid movement into the forming blastocoel. Within the trophectoderm of the bovine blastocyst, multiple isozymes of the Na-K-ATPase are expressed. Immunolocalization has demonstrated that the alpha1-isozyme localizes within the basolateral membrane, whereas the alpha 3-isozyme localizes to the apical cell margins. Gene-specific RT-PCR and wholemount indirect immunofluorescence confocal laser scanning microscopy were used to examine expression of the Na-K-ATPase gamma-subunit (a regulatory subunit of the Na-K-ATPase) throughout development of bovine preattachment embryos in vitro. Expression of mRNA transcripts for the gamma-subunit was detected throughout bovine pre-attachment development from the fertilized one-cell embryo to the blastocyst stage. A similar pattern of expression was also observed for gamma-subunit protein, and immunofluorescence was detected within the membranes of embryonic blastomeres at all stages of development. In contrast to the expression patterns observed for the alpha-subunits, gamma-subunit proteins were detected in both the basolateral and apical cell margins of the trophectoderm, and surrounding all cells of the inner cell mass. Co-localization studies demonstrated that gamma-subunit peptides are co-expressed with the alpha1-subunit in the basolateral domains of the trophectoderm. These results indicate a role for the gamma-subunit of the Na-K-ATPase in modulating Na(+)-pump activity in both apical and basolateral margins of the trophectoderm during formation and expansion of the bovine blastocyst, and adds a further level of complexity to Na(+)-pump regulation of cavitation.


http://www.reproduction-online.org/cgi/content/abstract/129/4/435

There is some evidence suggesting that Ca2+ is involved in processes that occur during the development and function of spermatozoa. Calcium-dependent proteins, such as calmodulin, are expressed during mammalian spermatogenesis further suggesting that Ca2+ takes part in its regulation. However, the precise roles of Ca2+ in spermatogenesis remain to be elucidated. Calpains are a family of Ca2+-dependent cysteine proteases whose members are expressed ubiquitously or in a tissue-specific manner. Calpain has been demonstrated to mediate specific
Ca2+-dependent processes including cell fusion, mitosis and meiosis. We herein followed the expression pattern of calpain's ubiquitous isoforms, 1 and 2, throughout spermatogenesis at the RNA and protein levels by RT-PCR and Western blotting analysis. Both RNA and protein studies revealed that these isoforms are expressed in all spermatogenic cells. The expression of calpain 1 levels is slightly higher in spermatocytes entering the meiotic phase. Both calpain isoforms are also expressed in mouse spermatozoa and are localized to the acrosomal cap. Inducing capacitated spermatozoa to undergo the acrosome reaction in the presence of a selective calpain inhibitor significantly reduced the acrosome reaction rate in a dose-dependent manner. Thus, calpain, a pluripotential protease with numerous substrates, may serve as an effector in more than one pathway in the complex process of spermatogenesis and in the events preceding fertilization, such as the acrosome reaction.


http://www.reproduction-online.org/cgi/content/abstract/128/4/463

Endothelium-derived endothelin-1 (ET-1) and nitric oxide (NO) are pivotal regulators of corpus luteum (CL) function. To have a better insight into their synthesis and action, members of the ET system (ET-1, ET converting enzyme (ECE-1) isoforms a-d, ETA and ETB receptors) along with NO synthase (NOS) isoforms - endothelial (e)NOS and inducible (i)NOS - were quantified in CL-derived endothelial cells (CLEC). The expression of these genes in microvascular CLEC, obtained by lectin-coated magnetic beads, was compared with cells removed from the luteal microenvironment and maintained in culture for different durations, and with endothelial cells (EC) derived from a large blood vessel (i.e. bovine aortic endothelial cells, BAEC). The profile of gene expression in the different EC types was determined by quantitative real-time PCR. Freshly isolated EC from mid-cycle CL exhibited high ET-1 receptor expression (both ETA and ETB), low ET-1 synthesizing ability (both prepro (pp) ET-1 and ECE-1), but elevated iNOS - the high throughput NOS isoform. The distinct phenotype of CLEC was lost soon after an overnight culture. ETA and ETB receptor levels declined, ppET-1 levels increased while iNOS was reduced. These changes were extenuated during long-term culture of CLEC. The general pattern of gene expression in BAEC and long-term cultured CLEC was similar yet some differences, reminiscent of freshly isolated CLEC, remained: ECE-1c, ETB receptor and NOS isoforms were expressed differently in BAEC as compared with lines of CLEC. This study suggests that the luteal microenvironment is necessary to sustain the selective phenotype of its resident endothelial cells. The inverse relationship between ppET-1 and iNOS observed in freshly isolated CLEC and in cultured cells is physiologically significant and suggests that ET-1 and NO may modulate the production of each other.


http://www.reproduction-online.org/cgi/content/abstract/124/3/353

It has been observed that apoptosis occurs in human blastocysts. In other types of cell, the characteristic morphological changes seen in apoptotic cells are executed by caspases, which are regulated by the BCL-2 family of proteins. This study investigated whether these components of the apoptotic cascade are present throughout human preimplantation development. Developing and arrested two pronucleate embryos at all stages were incubated with a fluorescently tagged caspase inhibitor that binds only to active caspases, fixed, counterstained with 4,6-diamidino-2-phenylindole (DAPI) to assess nuclear morphology and examined using confocal microscopy. Active caspases were detected only after compaction, at the morula and
blastocyst stages, and were frequently associated with apoptotic nuclei. Occasional labelling was seen in arrested embryos. Expression of proapoptotic BAX and BAD and anti-apoptotic BCL-2 was examined in single embryos using RT-PCR and immunohistochemistry. BAX and BCL-2 mRNAs were expressed throughout development, whereas BAD mRNA was expressed mainly after compaction. Simultaneous expression of BAX and BCL-2 proteins within individual embryos was confirmed using immunohistochemistry. The onset of caspase activity and BAD expression after compaction correlates with the previously reported appearance of apoptotic nuclei. As in other types of cell, human embryos express common molecular components of the apoptotic cascade, although apoptosis appears to be suppressed before compaction and differentiation.

Reproductive Toxicology (1)


http://www.sciencedirect.com/science/article/B6TC0-435CSWR-5/2/9f3b5782034320e7912b5a9a4928cad0

Butadiene diepoxide (BDE), a reactive metabolite of 1,3-butadiene that is an important industrial chemical used in synthetic rubber production causes a dose-dependent inhibition of deciduoma development in pseudopregnant Sprague-Dawley rats. This study used 4 daily i.p. BDE doses of 0.20, 0.25, 0.30, 0.35, or 0.40 to characterize mechanisms that may be responsible for the antideciduoma effect. Pseudopregnant rats were treated either before (pseudopregnancy [PPG] days 1-4) or after (PPG days 5-9) deciduoma induction by endometrial trauma with a blunt needle. Animals were killed on PPG day 9 and evaluated for serum progesterone and endometrial protein and DNA. RT-PCR was used to measure message for estrogen receptor (ER) [alpha] and pituitary adenylate cyclase-activating polypeptide (PACAP). Substrate zymography and Western blotting were used respectively to measure matrix metalloproteinase (MMP)-9 and inducible nitric oxide synthase. The antideciduoma effects of BDE were associated with decreases in endometrial weight, protein, and DNA, with decreases in serum progesterone, and with decreases in PACAP message and MMP-9. A reduction in NOS was identified at the highest dose of BDE. Message for estrogen receptor (ER) [alpha] was not affected at any dose. We conclude that the reduction in decidual proliferation was direct and appeared to be associated with either 1) a decrease in the effectiveness of the deciduogenic stimulation and/or a weakened endometrial sensitivity to the stimulus; or 2) an effect on deciduoma development. Molecular mechanisms that apparently contributed to BDE inhibition of decidual metabolism included the synthesis of protein and DNA involved in decidual growth, the synthesis and activation of a matrix metalloproteinase for degradation of the extracellular matrix that is essential for tissue remodeling during deciduoma development, and the nitric oxide/nitric oxide synthase and pituitary adenylate cyclase-activating peptide systems that are involved in promoting vasodilation and increased vascular permeability to enhance the availability of substrates for maximal deciduoma growth. The ovotoxicity of BDE, which has previously been established, may indirectly affect decidual proliferation by reducing progesterone, the preeminent endocrine regulator of deciduoma development. The findings also suggest that BDE may possess no estrogenic action since it was associated with endometrial weight loss and unaltered levels of the estrogen receptor [alpha] mRNA expression.

http://www.sciencedirect.com/science/article/B6VN3-47DDBM-1C/2/701a8333b92d149bcfac19d01fdeaaa

"Chelex 100" chelating resin has been previously proposed for the rapid extraction of human DNA for polymerase chain reaction. Protocols are given for the rapid extraction of bacterial and viral DNA from cultures or clinical samples. The DNA samples obtained were suitable for use in polymerase chain reaction.


http://www.sciencedirect.com/science/article/B6VN3-3V7PB37-9/2/959942c811704a88371e01edf07d6abc

In order to study a 19-kDa protein (p19) of Campylobacter jejuni, we purified this protein to homogeneity from C. jejuni strain 81176 by anion exchange chromatography. The molecular weight of the native protein is 19,000 daltons. P19 was found to be acidic with an isoelectric point of 4.8 and was located in the periplasmic space of the bacteria. The 20 N-terminal amino acids were sequenced and no significant similarities with known proteins were shown. A monoclonal antibody showed that p19 is conserved in the 2 species C. jejuni and C. coli. Analysis of sera from 23 patients with a Campylobacter-related infection indicated that p19 is not immunogenic during natural infection in man. The gene encoding p19 was cloned and no strong homologies with known sequences were identified. The preparation of a knockout mutant in p19 will enable the investigation of the function of this cell wall component of Campylobacter.


http://www.sciencedirect.com/science/article/B6VN3-3XXDWSF-3/2/133e5f862e14c8665a31fb87cfaa233d

The 16S-23S intergenic spacer regions of four Acinetobacter genomic species belonging to the A. calcoaceticus-A. baumannii (Acb) complex, i.e. genomic species 1 (A. calcoaceticus), genomic species 2 (A. baumannii), genomic species 3 and Tjernberg and Ursing (TU) genomic species 13, have been cloned and sequenced. Sequence analysis led to the discovery of a single copy of Ile and Ala tRNA genes within each spacer. Sequence comparison allowed the identification of a 192-base-pair long highly conserved sequence between the 3' end of the 16S rRNA and the 5' end of the tRNAAla genes. Moreover, two short regions, which were specific to, respectively, genomic species 2 and 3, could be identified. Oligonucleotides corresponding to these sequences were constructed and tested for the ability to hybridize with chromosomal DNA extracted from Acinetobacter belonging to different genomic species and with chromosomal DNA of other
bacterial genera. One of these oligonucleotides was demonstrated to be useful as a sensitive and specific probe for A. baumannii. A less sensitive probe for Acinetobacter genomic species 3 was also developed.


http://www.sciencedirect.com/science/article/B6VN3-3Y5FMKC-8/2/bffca24f68b2b5eb3602432c4911f1c

Random amplified polymorphic DNA (RAPD) analysis was applied to genomic DNA from nineteen yeast strains belonging to the genera Saccharomyces and Zygosaccharomyces. Results obtained with five primers indicated that this technique is a powerful tool for yeast differentiation and identification. The data were consistent with those derived from restriction fragment length polymorphism (RFLP) using two S. cerevisiae DNA probes. We conclude that RAPD fingerprinting, combined with the analysis of RFLP, can provide unambiguous type assignment in yeasts.


http://www.sciencedirect.com/science/article/B6VN3-41GWN7Y-9/2/10b1617605570d31fc47b17aa77edfab

Detection of human pathogenic viruses by molecular techniques might be suitable for identifying viral pollution in environmental waters and for improving diagnosis in patients. Environmental samples were taken from bathing areas and sewage treatment plants in southwestern France. Small volume samples (50 [mu]L) were tested. Five groups of enteric pathogenic viruses were studied: enteroviruses, Norwalk-like viruses (NLVs), hepatitis A virus, rotaviruses and adenoviruses. Moreover, human samples were tested for NLV. After extraction of viral nucleic acids (Boom's procedure), a nested polymerase chain reaction was conducted before hybridization. Five bathing waters out of 26 were positive for one viral group, without systematic association with bacterial contamination. Eight sewage plant samples out of 13 were positive for at least one viral group. Seven patients out of 45 were NLV-positive. Molecular techniques allow efficient screening of viral contamination in environmental waters and the study of NLV molecular epidemiology.


http://www.sciencedirect.com/science/article/B6VN3-3WB7RD8-C/2/01c1e3e7eeab351a96d8c6ec2c36ec84f

Bacteriophage [lambda] adsorbs to its Escherichia coli K12 host by interacting with a specific cell surface receptor, the outer membrane protein LamB. Previous genetic analyses led us to define a set of residues at the surface of LamB, which belong to the [lambda] receptor site. Further genetic studies indicated that the C-terminal portion of J, the tail fibre protein of [lambda], was directly
The present work describes first in vitro studies on the interactions between J and LamB. The J gene of \( \lambda \) was cloned into a plasmid vector under ptac promoter control and expressed in E. coli. We showed that J could be expressed at high levels (up to 28% of whole cell proteins), in an insoluble form. Anti-J antibodies, induced in rabbits immunized with insoluble J extracts, appeared to specifically neutralize \( \lambda \) infection. Under defined conditions of extraction, the J protein was obtained in a soluble form. We showed that solubilized J was able to interact with LamB trimers in vitro. Implications for future studies on the interactions between LamB and J are discussed.

**Research in Veterinary Science**  (7)


http://www.sciencedirect.com/science/article/B6WWR-48BC03B-1/2/0a4dcd9bb9df501470d11070e2d5bea

Chlamydia abortus-DNA was detected using a touchdown enzyme time-release (TETR)-polymerase chain reaction (PCR) assay as an improved test for sensitive and rapid diagnosis of abortion in small ruminants. Two hundred and fifty two placenta, liver or spleen tissue samples from aborting ewes and goats or aborted lambs and kids in which C. abortus infection was suspected were examined by TETR-PCR and the results were compared with cell culture. Sixty-five tissue samples were found to be TETR-PCR positive while only 56 samples were cell culture-positive. After resolution of discrepant samples with a confirmatory nested PCR assay, TETR-PCR had a sensitivity of 97% and a specificity of 99.5% while culture had a sensitivity of 84.8% and a specificity of 100%. The analytical sensitivity of the TETR-PCR assay was determined with DNA extracted from 4-fold serial dilution of C. abortus B577 culture and found to be 0.25 inclusion-forming unit per PCR. No reduction in the analytical sensitivity was noted when the assay was tested with mouse liver samples spiked with 4-fold serial dilution of C. abortus B577 culture. No target product was amplified when DNA from Chlamydia pecorum was tested. TETR-PCR used in this study is a practical, rapid, sensitive and specific assay that could be used for the detection of C. abortus in infected tissue samples. We recommend the use of this assay as a supplemental diagnostic tool for detection of C. abortus in infected tissue samples.


http://www.sciencedirect.com/science/article/B6WWR-4CWRXMS-1S/2/db52f8cdd300a9478561256682d0142f

The most important consequence of equine herpesvirus-1 (-1) infection is abortion. The object of the present study was to characterise further a murine -1 abortion model and to make comparisons with the natural host with particular reference to the stage of gestation during which the infection occurs. /c mice at different stages of pregnancy were infected intranasally with -1 (strain A134); they suffered respiratory distress, weight loss, and other constitutional signs of infection. When the virus was inoculated in the late second or early third week of gestation dead or dying fetuses were aborted, whereas infection between seven and nine days of pregnancy led
to fetal death and resorption. During the process of resorption, complications were observed. Virus was frequently isolated from the placentas and occasionally from the tissues of the aborting fetuses, depending on the severity of the infection of the placentas. In some cases, therefore, the inoculation resulted in abortion although the infection was restricted to the placenta. Virus antigen was detected in the placentas, lungs and occasionally in other tissues of the aborting fetuses. The potential of this murine model for testing methods for the diagnosis and control of equine abortion is discussed.


Cattle from Northern Portugal, many with pulmonary lesions typical of contagious bovine pleuropneumonia, were investigated for the presence of Mycoplasma mycoides subspecies mycoides small colony (MmmSC), which is the causative agent of CBPP, with several detection tests. Sandwich ELISA that included a culture enrichment stage, and 2 different PCR diagnostic systems were used to detect MmmSC in lung and mediastinal lymph node tissues from these animals. The comparison of typical CBPP pathology with the results of detection revealed that no single one of these methods provided a perfect match to the pathological data. Best performing tests were the PCR with laser induced fluorescence and PCR with pleuroTRAP kit (Chemicon, Australia), which are diagnostic systems based on amplification of genomic MmmSC DNA followed by sensitive detection of the amplified products. These were followed by the broth-enriched sandwich ELISA, which uses a monoclonal antibody specific to the M. mycoides cluster, to capture the antigen.


The tumour suppressor protein p53 enhances the genetic stability of the cell and plays a critical role in tumour suppression. Equine p53 was analysed by sequencing exons 5 to 9, a region which includes most known mutations and all the mutational hotspots in the species that have been investigated. The fragment was amplified, cloned and sequenced from genomic and complementary DNA. A comparison of the predicted amino acid sequences between the horse and other species resulted in identities between 66 per cent with the clawed frog and 92 per cent with the cat. Using the single strand conformation polymorphism technique, exons 5 to 8 amplified from sarcoid tissue and peripheral leucocytes of 28 sarcoid-affected and 11 healthy horses were screened for mutations. No mutations were identified, suggesting that the frequency of p53 mutations in equine sarcoid might be low. However, the high incidence of bovine papillomavirus infection in equine sarcoid may indicate the functional inactivation of p53 by -encoded E6 protein.

Davidson, A. J., J. E. Hodgkinson, et al. (2005). "Cytokine responses to Cyathostominae larvae in the
To investigate cytokine responses in cyathostomin infection, we quantified mucosal interleukin-4 (IL-4), interleukin-10 (IL-10), tumour necrosis factor (TNF)-alpha and interferon (IFN)-gamma by reverse transcriptase-competitive polymerase chain reaction. The analysis was performed on large intestinal wall samples obtained from six anatomical sites spanning the caecum and colon of 17 naturally exposed horses. The numbers of developing larvae (DL) and early third stage larvae (EL3) were ascertained using transmural illumination and pepsin digestion techniques, respectively. Levels of each cytokine transcript were correlated with local intestinal wall burdens of Cyathostominae larvae. IL-4 and IL-10 levels showed significant correlations with EL3 and DL burdens at several sites. No significant correlations were observed with IFN-gamma. A pro-inflammatory response, typified by detection of TNF-alpha transcript, was observed at a few sites in some horses with inflammatory enteropathy associated with emerging or emerged larvae. However, this cytokine was measured at an insufficient number of sites to enable statistical analysis. Levels of IL-4, IL-10 and IFN-gamma transcript were compared between two groups: one group consisting of horses with low to high mucosal burdens (Group A) and the other, of horses with negative/negligible mucosal burdens (Group B). Significant differences in IL-4 (P < 0.001) transcript levels were observed between the groups, with higher levels observed in Group A. No significant differences in IFN-gamma were observed. Taken together, these results indicate that Th2 responses predominate in mucosal Cyathostominae infection prior to larval reactivation.


Seven Swedish isolates of Ehrlichia species from the blood of four dogs and three horses with clinical granulocytic ehrlichiosis, were identified by direct solid phase DNA sequencing of polymerase chain reaction (PCR) products from the 16S rRNA gene. The amplified DNA fragments were produced with primers complementary to the universal regions, U1, U2, U5 and U8 of the 16S rRNA molecule. Identical sequences were obtained from all seven isolates. This nucleotide sequence was similar to the sequences deposited in GenBank for Ehrlichia phagocytophila and E equi. The sequence of the Swedish ehrlichiae differed in two nucleotide positions from the E phagocytophila sequence and in three positions from the E equi sequence, and it is tentatively proposed that it is a subspecies of one of these two. The alignment of the sequence of the Swedish isolates with a recently deposited sequence from human cases of ehrlichiosis in the USA revealed 100 per cent identity in a segment of about 1400 bp.

The virus isolation-immunoperoxidase test on cell cultures and the reverse transcription-polymerase chain reaction assay were compared for the detection of bovine viral diarrhoea virus directly in serum samples. Material for this study consisted of 403 sera originating from cattle in 41-infected Finnish dairy herds and one suckler cow herd. The presence of virus was demonstrated in 48 samples by both assays. In addition, two more samples were found to be positive by the assay. Both methods proved to be extremely sensitive, detecting pestiviruses even in high serum dilutions, and thus to be suitable for demonstrating the occurrence of persistently infected cattle. In conclusion, the method used had the advantage of ascertaining nucleic acid sequences in samples in which the virus had been inactivated, eg during transport or due to the presence of neutralising antibodies.

Respiration Physiology (1)


http://www.sciencedirect.com/science/article/B6T3J-3RSFWSR-8/2/1675acece557c33cefd7b3f1b960e27b

Brainstem serotonin (5-HT)-containing cells are remarkable for their widespread axonal projections and having their highest activity during wakefulness and lowest during rapid eye movement sleep. One important site of action of 5-HT is on upper airway motoneurons. However, which of the 14 known 5-HT receptors mediate the effects is uncertain. We used the reverse transcriptase/polymerase chain reaction to detect mRNA for six distinct 5-HT receptors (1A, 1B, 2A, 2C, 3 and 7) in 50 nl micro-punches collected from the hypoglossal (XII) motor nucleus and, for comparison, from the viscerosensory nucleus of the solitary tract (NTS) in adult rats. The relative abundance of the distinct mRNAs was characterized by the minimal number of amplification cycles (25-40) necessary to detect a given mRNA. In the XII nucleus, mRNA for type 1B, 2A and 2C receptors was detectable after 29-31 cycles, detection of type 3 and 7 receptor mRNA required 33-35 cycles; and type 1A receptor mRNA was not detected. In the NTS, detection of mRNA for type 1B, 2C and 7 receptors required 31-33 cycles; type 1A receptor mRNA required 39 cycles; and type 2A receptor mRNA was not detected. The data from the XII nucleus demonstrate that not only the previously recognized type 1B, 2A and 2C receptors, but also type 3 and 7 receptors have the potential to mediate serotonergic effects in XII motoneurons.

Respiratory Medicine (2)


http://www.sciencedirect.com/science/article/B6WWS-4CDJ15V-2/2/19320bdf9b2c4d9546cd3d7346d6453
Interleukin 4 (Il-4) is an immunoregulatory cytokine which induces T-cell proliferation and differentiation into a Th2 phenotype, and is of particular importance for the induction of IgE synthesis. In the present study, the capability of human peripheral blood eosinophils from allergic and non-allergic donors to produce Il-4 was examined. Using reverse transcribed polymerase chain reaction (RT-PCR), it was shown that highly purified eosinophils from allergic patients express mRNA for Il-4. Resting eosinophils also gave specific immunoreactivity with anti-Il-4 antibodies, consistent with translation of Il-4 mRNA. Light and electron microscopic immunocytochemistry revealed that Il-4 was prestored in the eosinophilic granules. These results were confirmed by Il-4 specific ELISA which showed that Il-4 production could be upregulated in the eosinophils and released from the eosinophils following stimulation with the calcium ionophore A23187. These data indicate that eosinophils may be an important source of Il-4 at sites of allergic inflammation. Thus, eosinophils may act as immunomodulatory cells enhancing the allergic response through formation of Th2-cells and inducing the isotype switching to IgE in human B-cells.


http://www.sciencedirect.com/science/article/B6WWS-4BKN11H-1/2/1c543489788fe1a5d2b3e8c6b7cc7265

A multiplex polymerase chain reaction (PCR) was developed for the simultaneous detection of Chlamydia pneumoniae, Mycoplasma pneumoniae and Legionella pneumophila. Oligonucleotide primers for the amplification of the DNA of these three organisms were optimized for use in combination in the same reaction. PCR products were detected by the Micro-Chip Electrophoresis Analysis System. Clinical samples were obtained from 208 community-acquired pneumonia (CAP) patients who were participants in a multicenter CAP surveillance study performed at seven medical schools and their affiliate hospitals in Japan. No significant differences in the sensitivity of each primer set were observed when tested in both the multiplex and monoplex PCR assays. Our multiplex PCR was able to reliably detect 10 copies/100 [mu]l of each of the three pathogen DNAs. Of the panel of 208 samples, 14 of 15 C. pneumoniae, 10 of 10 M. pneumoniae, eight of eight L. pneumophila and 165 of 176 negative samples were correctly identified. Eleven cases who were the multiplex PCR positive and conventional method negative were observed. The PCR findings were of possible significance in at least four of these patients. Our multiplex PCR assay could potentially be used as a diagnostic and epidemiological tool. Further prospective studies are needed to establish its clinical usefulness.

Review of Palaeobotany and Palynology (1)


http://www.sciencedirect.com/science/article/B6V6W-45H0B79-4/2/491b7257f4036364923180f52524d8a2

Schizaeaceae fossils have been documented throughout Mesozoic and Cenozoic deposits, but
our understanding of this fossil record is hampered by uncertainties with respect to the relationships of living species. To start building a phylogenetic framework for the family, an initial phylogenetic analysis of living species using plastid rbcL nucleotide sequence data is conducted. The analysis supports Schizaea s. lat. and Lygodium monophyly, but Anemia is resolved as paraphyletic to Mohria. In the Anemia/Mohria clade, monophyly of subgenus Anemiorrhiza is supported, but Coptophyllum is resolved as paraphyletic to subgenus Anemia. In Schizaea s. lat., both Schizaea s. str. and Actinostachys are well supported and Microschizaea is grouped with Schizaea s. str., although only one Microschizaea species (Schizaea pusilla) was included. These results are largely congruent with previous morphology-based analyses. In Lygodium however, results presented contrast with recent morphological analyses highlighting the problems of identifying Lygodium subgeneric groups. Using the resulting phylogeny as a framework, putative relationships of fossil species are discussed, tentative minimum age estimates for generic crown group diversifications are made, and possible conclusions with respect to the origins of habit and habitat preferences are discussed. The fossil evidence indicates that subgeneric groups within the Anemia/Mohria clade are comparatively ancient, originating during the Early Cretaceous, and the putative placement of fossil Anemia within the crown group of living subgenus Anemiorrhiza would indicate that their calcareous habitat preference may be a relic feature that has persisted ever since the Early Cretaceous. Lygodium on the other hand appears to have passed through a diversity bottleneck. Modern species diversity probably originated in the Neogene, and the earliest fossil evidence for the origin of their vining and trailing habit comes from the placement of Miocene fossil Lygodium within the crown group of living species.

Reviews in Molecular Biotechnology (1)


http://www.sciencedirect.com/science/article/B6VR0-44VW8G-8/2/70bb7db05733e41574524ec8fdff8285

High throughput screening, increased accuracy and the coupling of real-time quantitative PCR (Q-PCR) to robotic set-up systems are beginning to revolutionise biotechnology. Applications of Q-PCR within biotechnology are discussed with particular emphasis on the following areas of biosafety and genetic stability testing: (a) determination of the biodistribution of gene therapy vectors in animals; (b) quantification of the residual DNA in final product therapeutics; (c) detection of viral and bacterial nucleic acid in contaminated cell banks and final products; (d) quantification of the level of virus removal in process validation viral clearance studies; (e) specific detection of retroviral RT activity in vaccines with high sensitivity; and (f) transgene copy number determination for monitoring genetic stability during production. Methods employed for Q-PCR assay validation as required in ICH Topic Q2A Validation of Analytical Methods: Definitions and Terminology (1st June 1995) are also reviewed.

Revue Francaise des Laboratoires (1)

http://www.sciencedirect.com/science/article/B6VRF-46SVDDB-9T/2/ed22165554b2e41f0a5b621ff1a7a462

Resume
L'epidemiologie des souches de Klebsiella pneumoniae productrices de [beta]-lactamase a spectre etendu (KpBLSE), isolees durant une periode de 16 mois dans un service de reanimation, a ete etudiee. Un programme associant le renforcement des mesures d'isolement, le depistage systematique des patients a l'admission puis une fois par semaine et une decontamination digestive (DDS) a ete instaure en 1992. Afin d'analyser les transmissions croisees, differents marqueurs phenotypiques et genotypiques (contenu plasmidique, profils d'ADN total restreint par electrophorese en champ pulse, rep-PCR) ont ete utilises pour comparer 138 souches de KpBLSE isolees chez 64 patients. L'incidence des colonisations et/ou des infections etait de 11,9%. Cinquante cinq cas ont ete consideres comme acquis dans l'unite et 9 cas ont ete importes. Parmi les 45 infections observees chez 32 patients, les infections urinaires ont ete les plus frequentes. L'utilisation d'une DDS n'a pas permis de reduire l'acquisition de KpBLSE. L'utilisation combinee de plusieurs marqueurs s'est averee necessaire pour la differenciation des souches. Un clone producteur de [beta]-lactamase de type SHV-4 a ete a l'origine de 85% des cas acquis dans l'unite. Des cas sporadiques lies a des souches de KpBLSE productrices de [beta]-lactamases variees (TEM-3, SHV-2, SHV-3 et SHV-5) ont ete observes.

Rheumatology (5)


http://rheumatology.oupjournals.org/cgi/content/abstract/42/8/969

Objective. To assess the role of polymorphisms of the tumour necrosis factor (TNF) receptors, TNF-RI (p55) and TNF-RII (p75) in the susceptibility to and severity of rheumatoid arthritis (RA) in Dutch patients. Methods. A total of 319 consecutive RA patients, and a cohort of 90 female RA patients with detailed 12-yr follow-up were genotyped for the TNF-RI exon 1 (+36 A to G) and TNF-RII 3' UTR (+1690 T to C) polymorphisms. Results. The frequencies of the TNF-RI and TNF-RII polymorphisms were determined in both patient groups and healthy controls, but no significant differences were found. To determine the relationship of these polymorphisms to disease severity, the extent of joint damage in the cohort of 90 female RA patients was analysed. No differences in severity were observed. Conclusion. These TNF-RI and TNF-RII polymorphisms were not found to be associated with susceptibility to or severity of RA in the Dutch population.


http://rheumatology.oupjournals.org/cgi/content/abstract/41/4/445
Objectives. Recent studies have suggested that infective agents may be involved in the pathogenesis of giant cell arteritis (GCA), in particular Chlamydia pneumoniae and parvovirus B19. We investigated temporal arteries from patients with GCA for these infections as well as human herpes viruses using the polymerase chain reaction (PCR). Methods. Thirty temporal artery biopsies from 30 patients suspected of having GCA within a period of 1 yr were examined. Thirteen patients had classical GCA, two had biopsy-negative GCA, 10 patients had polymyalgia rheumatica and five patients had other conditions. DNA was extracted from frozen biopsies and PCR was used to amplify genes from Chlamydia pneumoniae, parvovirus B19 and each of the eight human herpes viruses: herpes simplex viruses HSV-1 and 2, Epstein-Barr virus, cytomegalovirus, varicella zoster virus and human herpes viruses HHV-6, -7 and -8. Results. In all 30 biopsies, PCR was negative for DNAs of parvovirus B19, each of the eight human herpes viruses and C. pneumoniae. Conclusions. We found no evidence of DNA from parvovirus B19, human herpes virus or C. pneumoniae in any of the temporal arteries. These agents do not seem to play a unique or dominant role in the pathogenesis of GCA.


http://rheumatology.oupjournals.org/cgi/content/abstract/43/10/1292

Objective. To identify potential mutations in the tumour necrosis factor receptor superfamily 1A gene (TNFRSF1A) in a Japanese female patient with recurrent fever complicated by systemic lupus erythematosus (SLE), and in her family members. Methods. DNA sequencing of exons 1-10 of the TNFRSF1A gene was performed to determine mutations that might be associated with the tumour necrosis factor receptor-associated periodic syndrome (TRAPS). Moreover, the TNFRSF1A gene was examined in Japanese patients with autoimmune diseases, including SLE, rheumatoid arthritis (RA), mixed connective tissue disease (MCTD) and Behcet's disease, and in healthy Japanese controls. Enzyme-amplified sensitivity immunoassay (EASIA) analysis was used to assess serum levels of TNF, the 55-kDa TNF receptor (TNFRSF1A) and the 75-kDa TNF receptor (TNFRSF1B). Membrane TNFRSF1A expression was analysed on the surface of peripheral blood mononuclear cells by flow cytometry. Results. A novel mutation, a heterozygous C to T transition in exon 3 which substitutes an isoleucine for a threonine at position 61 (T61I) was detected in the TNFRSF1A gene derived from the genomic DNA of a Japanese female TRAPS patient. Two nieces and one nephew, all with a similar clinical phenotype, also possessed the same TNFRSF1A mutation. We further demonstrated the same mutation in five of 60 SLE patients (8.3%) and in five of 120 healthy individuals (4.2%), with no significant differences. Although high titres of serum TNF and soluble TNFRSF1B protein were observed in this patient, low titres of soluble TNFRSF1A protein were detected. However, a defect in TNFRSF1A shedding in vitro was not observed in monocytes derived from this patient. Conclusion. This is the first report of a TRAPS patient associated with SLE with a novel TNFRSF1A mutation (T61I).


http://rheumatology.oupjournals.org/cgi/content/abstract/42/8/980

Objective. We investigated the association of gene polymorphisms in APRIL, a new member of the TNF family, with systemic lupus erythematosus. Methods. To detect polymorphisms of the human APRIL gene by exon-specific polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis, we first determined the structure of the human APRIL gene.
We designed exon-specific oligonucleotide primers according to the genomic DNA sequence of APRIL. All of the coding regions in exons of the APRIL gene were analysed by exon-specific PCR-SSCP in 148 SLE patients and 146 unaffected controls, then the nucleotide sequences of exons that displayed aberrant bands were determined. Results. The human APRIL gene comprised at least six exons with five introns, spanning approximately 2.8 kilobases of the genomic DNA. By exon-specific PCR-SSCP, we identified two novel polymorphisms at codons 67 and 96. Both had amino acid substitutions: G67R and N96S respectively. Only the 67G allele was associated with SLE in 148 Japanese SLE patients, with allele frequency 0.662 compared with 0.575 for 146 unaffected controls (P=0.0302). The frequency of the individuals who possessed at least one 67G allele in SLE patients (91.9%) was significantly higher than that in the unaffected controls (80.1%) (P=0.0036). Conclusion. The 67G allele of APRIL may be a contributing factor in the pathogenesis of SLE.


http://rheumatology.oupjournals.org/cgi/content/abstract/43/11/1357

Objectives. Overproduction of interleukin-10 (IL-10) is a pivotal feature in the pathophysiology of systemic lupus erythematosus (SLE). In vitro IL-10 secretion has previously been related to haplotypes of the IL-10 promoter microsatellite polymorphisms IL10.R and IL10.G. Published data concerning the association of IL10.G alleles with susceptibility to SLE are inconsistent in different ethnic populations. We analysed the association of IL-10 promoter microsatellite polymorphisms with disease susceptibility and manifestations in German Caucasian patients with SLE. Methods. Two hundred and ten (210) SLE patients fulfilling the 1997 revised ACR criteria and 158 ethnically, age- and sex-matched healthy controls were genotyped for the IL-10 promoter microsatellite polymorphisms by fragment length analysis. Haplotypes were reconstructed using a Bayesian coalescent theory-based method with PHASE software. Allele and haplotype distributions were compared between patients and controls and between subgroups of patients with different clinical and immunopathological findings. Results. In the study population no significant associations of individual IL10.R and G alleles or their haplotypes with susceptibility to SLE or major clinical manifestations were observed. By contrast, alleles G14 and G15 and haplotypes R2-G14 and R2-G15 were significantly over-represented in anti-Sm antibody-positive patients. Conclusions. The IL-10 promoter microsatellite polymorphisms and their haplotypes do not constitute a major risk factor for SLE in German Caucasians. However, the identification of genetic markers such as the IL-10 high-response haplotype R2-G14 predisposing for the production of anti-Sm antibodies may help to elucidate the conditions that lead to the development of SLE.

RNA (3)


http://www.rnajournal.org/cgi/content/abstract/8/1/16

http://www.rnajournal.org/cgi/content/abstract/11/3/308

The yeast long terminal repeat (LTR) retrotransposon Ty1, like retroviruses, encodes a terminally redundant RNA, which is packaged into virus-like particles (VLPs) and is converted to a DNA copy by the process of reverse transcription. Mutations predicted to interfere with the priming events during reverse transcription and hence inhibit replication are known to dramatically decrease transposition of Ty1. However, additional cis-acting sequences responsible for Ty1 replication and RNA dimerization and packaging have remained elusive. Here we describe a modular mini-Ty1 element encoding the minimal sequence that can be retrotransposed by the Ty1 proteins, supplied in trans by a helper construct. Using a mutagenic screening strategy, we recovered transposition-deficient modular mini-Ty1-HIS3 elements with mutations in sequences required in cis for Ty1 replication and integration. Two distinct clusters of mutations mapped near the 5'-end of the Ty1 RNA. The clusters define a GAGGAGA sequence at the extreme 5'-end of the Ty1 transcript and a complementary downstream UCUCUC sequence, 264 nt into the RNA. Disruption of the reverse complementarity of these two sequences decreased transposition and restoration of complementarity rescued transposition to wild-type levels. Ty1 cDNA was reduced in cells expressing RNAs with mutations in either of these short sequences, despite nearly normal levels of Ty1 RNA and VLPs. Our results suggest that the intramolecular interaction between the 5'-GAGGAGA and UCUCUC sequences stabilizes an RNA structure required for efficient initiation of reverse transcription.


http://www.rnajournal.org/cgi/content/abstract/10/4/747

Study of early and transient response gene expression is important for understanding the mechanisms of response to growth stimuli and exogenous agents such as microbes, stress, and radiation. Many of the cytokines, proto-oncogenes, and other transiently expressed gene products are encoded by mRNAs that contain AU-rich elements (AREs) in their 3' untranslated regions (UTRs). In this article, we describe an approach to selectively synthesize ARE-containing cDNA (ARE-cDNA) using an innovative combination of culture treatment, thermostabilization of reverse transcriptase (RT) by the disaccharide trehalose, and use of optimized ARE-specific oligomers. The monocytic cell line, THP-1, was treated with cycloheximide and endotoxin to enrich for ARE-mediated gene expression followed by the RT procedure. Selection of ARE-cDNA with simultaneous suppression of abundant cDNA was made possible using the procedure as monitored by the preferential expression of IL-8, an ARE-cDNA molecule, over the abundant housekeeping cDNA, {beta}-actin. The use of trehalose dramatically reversed cDNA abundance, resulting in almost complete suppression of housekeeping cDNA. Finally, construction of specialized ARE-cDNA libraries confirmed the selectivity of ARE-cDNAs and the presence of rare genes. The ability to reverse the abundance of housekeeping and other highly expressed genes toward ARE genes facilitates the discovery and study of rare early response and transiently expressed genes.

http://www.sciencedirect.com/science/article/B6TC2-4CVX6Y2-1/2/47cf7f62ff4557b67d6575d0c2be1deb

The close homolog of L1 (CHL1), located on human chromosome 3p26.1, is a newly identified member of the L1 family of cell adhesion molecules which play important roles in cell migration, axonal growth, and synaptic remodeling. A positive association has been reported between a missense polymorphism in CHL1 gene and schizophrenia in the Japanese population [Sakurai, K., Migita, O., Toru, M., Arinami, T., 2002. An association between a missense polymorphism in the close homologue of L1 (CHL1, CALL) gene and schizophrenia. Mol. Psychiatry 7, 412-415]. An association between a missense polymorphism in the close homologue of L1 (CHL1, CALL) gene and schizophrenia. In order to test this finding, we genotyped four SNPs in the gene in the Han Chinese population using a sample of 560 cases and 576 controls. Analysis of allele frequencies in both samples also showed strong association between SNP rs2272522 (the same marker studied by K. Sakurai) and the disease (X2=31.591, PCHL1 gene and schizophrenia and indicate that CHL1 may be involved in the etiology of schizophrenia.


http://www.sciencedirect.com/science/article/B6TC2-4233NBT-5/2/ac0a993e99569ff2de5f27e81e294b34


http://www.sciencedirect.com/science/article/B6TC2-45RCDJT-66/2/3e31a09c3e949aca5ec16098b4e5bd07

There is growing evidence that some genetic predisposition is important in the etiology of schizophrenia. We have sought to implicate a major gene by performing a candidate gene association study comparing the allele frequencies of seven restriction fragment length polymorphisms (RFLPs) at six loci in both a psychiatrically normal control group (N = 51) and an affected (schizophrenia or schizoaffective disorder) group (N = 55). Each group comprised Caucasians of northern European origin. The candidate areas (D5S39, D5S78, dopamine receptor D2 (DRD2), D11S29, porphobilinogen deaminase (PBGD), and D11S84) were selected on the basis of prior cytogenetic findings in schizophrenics, linkage studies, and/or implicated gene products. The presence of a polymorphic ApaLI site within the PBGD gene showed a significant association with the presence of illness (P = 0.02). The relative risk of possessing the allele with the ApaLI site was 2.10. No significant association was found with any of the six other RFLPs. Our data suggests that either the PBGD gene itself or an unknown gene linked to and/or in linkage disequilibrium with the PBGD locus predisposes some individuals to schizophrenia. Independent replication of these findings will be required to determine their relevance to schizophrenia.
An association of HLA-DR8 and DR1 with DSM-III schizophrenia has been reported in Japan (Miyanaga et al. (1984) Biol. Psychiatr. 19, 121-129). To further investigate this preliminary finding, we compared HLA-DR types in 44 unrelated Japanese schizophrenics (DSM-III-R) with those in 51 unrelated, healthy Japanese volunteers. Group-specific PCR amplification was used in the determination of HLA-DR in the patients. No significant difference was observed in the frequency of any DR types between patients and controls, after statistical correction for multiple testing. However, the frequency of DR1 in our patients (23%) and controls (10%) was almost the same as those in the previous report (22% vs. 10%), which means that there is a suggestive trend which could become significant if numbers were larger. It is argued that an exact determination of HLA-DR by DNA typing is important in current HLA studies of schizophrenia.

Dysfunction of the gene for the NR1 subunit of the N-methyl-aspartate (NMDA) receptor (GRIN1) has been implicated in the pathogenesis of schizophrenia. In support of this hypothesis are behavioral abnormalities reminiscent of schizophrenia in mice with an attenuated expression of the NR1 subunit receptor and the reduced level of NR1 mRNA in postmortem brains of patients with schizophrenia. We screened single nucleotide polymorphisms (SNPs) in the upstream region between +51 and -941 from the translation initiation codon of GRIN1 and identified 17 SNPs, 10 of which were located within the region containing the Sp1 motif and the GSG motifs. As genotyping of 191-196 Japanese patients with schizophrenia and 202-216 controls revealed no significant association between schizophrenia and the SNPs in the upstream region of GRIN1, these SNPs apparently do not play a critical role in the pathogenesis of schizophrenia in the Japanese population.

Neurotransmitter-based hypotheses have so far led to only moderate success in predicting new pathogenetic findings in etiology of schizophrenia. On the other hand, the more recent oligodendroglia hypotheses of this disorder have been supported by an increasing body of evidence. For example, the expression level of the myelin associated glycoprotein (MAG) gene has been shown to be significantly lower in schizophrenia patient groups compared to control groups. Such an effect might be a result of genetic variations of the MAG gene. In order to test this hypothesis, we genotyped four markers within the MAG locus in 413 trios sample of the Han Chinese using allele-specific PCR. None of the four markers revealed noticeable allelic
significance. However, the four-marker and two-marker haplotypes covering components rs720309 and rs720308 were observed to be significantly associated with schizophrenia (PP=0.0001). The results demonstrated MAG might play a role in genetic susceptibility to schizophrenia. Furthermore, our finding of a possible association between the MAG locus and schizophrenia is in agreement with the hypotheses of oligodendritic and myelination dysfunction.

Science (2)


http://www.sciencemag.org/cgi/content/abstract/295/5563/2267


http://www.sciencemag.org

Scientia Horticulturae (8)


http://www.sciencedirect.com/science/article/B6TC3-3W3FGSM-K/2/f048dc1effe2481fa8515af15bd7d9f6

A procedure for the rapid extraction of partially purified nucleic acid extracts from Alstroemeria cultivars and the corresponding polymerase chain reaction (PCR) protocol for the generation of random amplified polymorphic DNA (RAPD) markers were established. Nucleic acid extracts from 23 Alstroemeria cultivars were amplified with 8 random decamers by PCR. OPC02, OPC03, OPD02, OPD05, OPD08, OPD11, OPD13 and OPD18 produced 24, 19, 21, 20, 10, 17, 25 and 29 RAPD bands, respectively. The distinctive RAPD patterns generated from these cultivars could be used as genomic 'fingerprints' to establish the identity of a given genotype. The 'Orchid' and 'Butterfly' types were clearly separated in distinct subclusters in a phylogram obtained by unweighted pair group method analysis (UPGMA) of the genetic distances. The 'Hybrid' types were distributed in two major subclusters, reflecting the diversity of the parental species used to generate the population. This phylogram conformed to expectations based on the available pedigree data.

http://www.sciencedirect.com/science/article/B6TC3-41B75WY-V/2/21027b4c6e48be7c48df0076289107d9

The aim of this study was to help establish the phylogenetic relationships between the wild taxa of the Brassica oleracea complex using a random amplified polymorphic DNA (RAPD) assay and also to test the potential use of RAPDs in discriminating among closely related species. A total of 22 populations belonging to 15 taxa were analysed; 20 arbitrary primers were studied and six were selected for the detection of 129 reproducible polymorphic fragments, ranging from 200 to 2400 base pairs. These genetic markers, which allowed us to distinguish the different taxa, were used to study the phylogenetic and evolutive relationships of the wild Brassica (n = 9) species. The dendrogram obtained reflects the already accepted genetic relationships among the 15 taxa. Three clearly separated branches are shown: the Western group, the Sicilian group and the Aegean group. The maximum diversity is detected in the Aegean group and the highest similarity coefficient is shown by the Sicilian group, results which are consistent with previous phytochemical analyses.


http://www.sciencedirect.com/science/article/B6TC3-4DXT80H-1/2/929900307236f64381a06663ecfa3fef

Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic stability of 10 micropropagated plants regenerated through axillary buds of clonal apple (Malus pumila Mill.) rootstock MM106. Eleven random decamer primers were successfully used to analyse genomic DNA from mother plants and in vitro plant material. A total of 129 scorable fragments were amplified with an average of 11.73 bands per primer. Among them, 99 were monomorphic and 30 were polymorphic with 23.2% polymorphism. Among these 30, 12 were found monomorphic across seven plants and parent. Three plants could be regarded as off-types. Our results show that RAPD markers could be used to detect the genetic similarities and dissimilarities in micropropagated material.


Twenty-five accessions of apple, representing eight cultivars (‘Golden Delicious’, ‘Delicious’, ‘Gala’, ‘Jonagold’, ‘Jonathan’, ‘Florina’, ‘Fior di Cassia’, and ‘Imperatore Dallago’) have been characterized with Random Amplified Polymorphic DNAs (RAPD). The reliability of the method was tested by analyzing separate scions of the same clone and also by comparing different accessions of the same cultivar. Using two separate ten bp primers, it was possible to obtain a distinctive fingerprint for each of the cultivars. The method is simple, rapid and should provide a useful system for documenting the identity of clonal material.

Eighteen cultivars of cocoyam (*Xanthosoma* spp.) and two cultivars of taro (*Colocasia esculenta* (L.) Schott) from the USDA/ARS germplasm collection were evaluated for genetic relatedness using RAPD data. Seven random primers generated 40 RAPD loci. Of the 18 cultivars screened, 11 (61%) were identical at all RAPD loci evaluated. A similarity matrix was constructed on the basis of the presence or absence of bands. Among cocoyam cultivars the genetic similarity ranged from 0.86 to 0.97 with a mean of 0.91. Cluster analysis identified two main clusters with some unexpected groupings. These data indicate that very little genetic variation exists within the accessions used in this study and that this *Xanthosoma* spp. collection is of limited value as a genetic resource.


We obtained information on the genetic relationship in wild *Gladiolus* species through randomly amplified polymorphic DNA (RAPD) analysis. Out of the 140 tested primers, 32 amplified a total of 133 RAPD bands in 33 *Gladiolus* species. The genetic distance was calculated from the data of these RAPDs, and a dendrogram was generated. Interspecific crosses were carried out in seven combinations within or between clusters, and F1 seedlings were obtained from most combinations. The RAPD analysis showed that these F1 seedlings were real hybrids. The results suggest that RAPD markers are useful for detecting genetic relationships in *Gladiolus* species, and for interspecific crosses in breeding programs.


Transformation of spray-type chrysanthemum was performed using *Agrobacterium tumefaciens* strain C58 and MP90 harboring a rice chitinase gene (cDNA clone named: RCC2). Eleven transgenic lines expressing the RCC2 gene were obtained. These lines showed enhanced resistance to gray mold (*Botrytis cinerea*), although the levels of resistance varied among the transgenic lines. Three higher resistance lines, Y12, Y61 and Y97, showed very slight symptoms against *B. cinerea* infection and which did not spread even if the incubation period was extended. In these three lines, a higher production of RCC2 protein was detected by enzyme-linked immunosorbent assay (ELISA) compared with non-transgenic plants. These results suggest that the RCC2 gene can be a useful tool to improve resistance to gray mold in chrysanthemum.
RAPD and microsatellites are used in the present study as molecular markers for characterisation of grapevine germplasm material. The studied varieties were located in two germplasm banks in Spain. Thirty nine accessions were included in the study, including some presumed synonymies and homonymies. Sixty six primers were used for the RAPD study. Twenty were selected for the multivariate analysis and grouping of the varieties. Seven varieties had a monotypic pattern. Twenty three accessions were also analysed with microsatellites with the VVMD7, VVS2, VVS5 and VVS29 loci, obtaining eight different patterns. The high discriminating ability of the RAPD analysis allowed all the studied varieties to be distinguished. A good concordance was obtained for both RAPD and microsatellites when the two analysis were carried out. The following synonymies were confirmed: Moristell and one of the accessions of Monastel; Maturana and Ribadavia; Concejon and one of the accessions of Monastrell; and most of the studied muscat varieties. Homonymies were detected for Miguel de Arco, Monastel, Monastrell and Turruttes. Both RAPD and microsatellites are considered as adequate molecular markers for characterisation of germplasm banks with the use of one or the other depending on the objectives of the study. When results are intended to compare with other laboratories or germplasm banks, microsatellites give simple and more comparable results.


ObjectivePost-streptococcal reactive arthritis (PSReA) may be a variant of acute rheumatic fever (ARF), but there still is debate on the relationship between the 2 entities. Possible associations with HLA class II antigens of PSReA (DRB1*01) and ARF (DRB1*16) were described previously in white Americans. To confirm these findings, we studied DRB1 alleles in a group of Italian children with PSReA and ARF. MethodsWe performed low-resolution HLA-DRB1 typing by a sequence-specific primer polymerase chain reaction method in 33 children with PSReA and 25 children with ARF. We also compared the DRB1 genotypes of our patients with 200 normal subjects from the same geographic area and typed in the same laboratory with the same methods. ResultsThe allele distributions at the DRB1 locus observed in PSReA patients, ARF patients, and controls were not significantly different from each other (chi-square test with small numbers, P = .65). The positivity for each of the 13 HLA-DRB1 alleles was compared in disease groups (PSReA and ARF) and controls, and failed to show any significant association. Comparisons of the frequency of the DRB1*01 allele among PSReA, ARF, and controls did not show any statistical differences. No significant difference in the frequency of DRB1*16 was present between ARF vs the control group, between ARF vs PSReA, and in PSReA patients when compared with controls. ConclusionsOur data do not confirm in Italian patients the previously reported associations of DRB1*01 and DRB1*16 with PSReA and ARF, respectively.

http://www.sciencedirect.com/science/article/B6THG-4B3JT2Y-1/2/d3b62fa144d94b26897d69b0e27305da

We present a SU-8 based polymerase chain reaction (PCR) chip with integrated platinum thin film heaters and temperature sensor. The device is fabricated in SU-8 on a glass substrate. The use of SU-8 provides a simple microfabrication process for the PCR chamber, controllable surface properties and can allow on chip integration to other SU-8 based functional elements. Finite element modeling (FEM) and experiments show that the temperature distribution in the PCR chamber is homogeneous and that the chip is capable of fast thermal cycling. With heating and cooling rates of up to 50 and 30 [deg]C/s, respectively, the performance of the chip is comparable with the best silicon micromachined PCR chips presented in the literature. The SU-8 chamber surface was found to be PCR compatible by amplification of yeast gene ribosomal protein S3 and Campylobacter gene cadF. The PCR compatibility of the chamber surfaces was enhanced by silanization.


A new thermally actuated valving concept using paraffin as single-use valving material was developed. The paraffin undergoes a phase transition in response to changes in temperature. A variety of single-use paraffin-based microvalves, including "close-open," "open-close-open," "T," and toggle designs, were demonstrated. Fluidic experiments showed that these microvalves had zero leakage and a maximum hold-up pressure of 40 psi in a "closed" position. A DNA polymerase chain reaction microdevice containing paraffin-based microvalves to enclose the sample solution in the reaction chamber during the thermal cycling was demonstrated. The paraffin-based microvalving technique has advantages over many existing active microvalve approaches, including a simple design, ease of fabrication, low cost, and ease of integration into complex microfluidic systems. Moreover, this technique is particularly attractive for single-use and disposable microfluidic devices.

http://www.sciencedirect.com/science/article/B6THH-4BHCMJ5-8/2/d8cafa127afa7cf1e607cc519ccd8039

This paper reports the development of epoxy microcasting technologies for the fabrication of plastic microfluidic platforms. Two new techniques one involving embedding active silicon devices in plastic microsystems using a polymer flip chip process and another involving surface micromachining to build active components like actuators for use in applications like pumping are discussed and explained. Application devices were fabricated for polymerase chain reaction (PCR) and capillary electrophoresis (CE).


http://www.sciencedirect.com/science/article/B6THH-4534CH9-1/2/a11eed4240d2fb663113e9e986293188

A microchannel chip for continuous-flow polymerase chain reaction (PCR) was developed using transparent materials. The microchannel was fabricated on a quartz glass substrate using standard photolithography and wet-etching techniques and was sealed by another quartz glass substrate. Two indium-tin-oxide (ITO) films were deposited on the etched substrate as a thermal source. To confirm the temperature distribution in the microchannel, we measured the fluorescence spectra of an aqueous solution of 1-pyrenesulfonic acid sodium salt (PS-Na), which is a temperature-indicator dye, in the microchannel under a continuous solution flow. The results confirm that the temperature distribution on the microchannel's ITO films was almost uniform (within +/-2 [deg]C) under two flow rates (56 and 152 nl/min). The slightness of this deviation indicates that the ITO films integrated into the microchannel chip can be very useful as a thermal source for PCR. An amplification of a 450 bp segment of Escherichia coli HB101 was successfully performed by two-stage (94 and 67 [deg]C) thermal cycling on the chip device.


http://www.sciencedirect.com/science/article/B6TC4-4C82FVF-4J/2/f0a4e01d90393a4ac1683e5cc5d4c260

In this retrospective study a total of 404 stools kept at -70[deg]C were tested for the presence of verotoxin-producing Escherichia coli (VTEC) by the polymerase chain reaction (PCR). Thirteen positive samples from 11 patients were identified by PCR which correlated with previous isolation of E. coli O157:H7. There was no failure to detect VTEC by PCR but PCR did not identify further VTEC isolates. We concluded that (a) the occurrence of VTEC other than serotype O157:H7 is rare in our demographic area, (b) PCR is effective in the identification of E. coli O157:H7, and (c)
PCR has the additional advantage over conventional culture methods of identifying VTEC, including sorbitol-fermenting serotypes, which might have been detected if we had extended our sample size.


http://www.sciencedirect.com/science/article/B6TC4-40079HF-B/2/286da697c44561a29832bea389877318

The nested polymerase chain reaction (PCR) was clinically investigated to detect the mip gene of Legionella species. PCR detected 20 clinically important Legionella species, such as L. pneumophila, L. micdadei, and L. bozemanii. Eight species of Gram-positive and -negative bacteria other than Legionella species were negative for PCR. The sensitivity of PCR determined by the detection limit of DNA quantity was 1.0 pg for the first PCR, but the sensitivity increased approximately 100-fold to 10 fg following the second PCR. The nested PCR was applied to detect L. pneumophila in the sputum and pleural effusion fluid obtained from a patient with Legionnaires' disease. Both fluids were PCR positive, but culture was negative for L. pneumophila. Our results indicated that the nested PCR may be a useful tool for the rapid detection of L. pneumophila and the clinical diagnosis of Legionnaires' disease.

Sex. Transm. Inf. (4)


http://sti.bmjjournals.com/cgi/content/abstract/79/5/393

Objectives: DNA amplification techniques have become widely used for the diagnosis of sexually transmitted infections. For the detection of Trichomonas vaginalis, PCR techniques are not yet widely used despite the publication of several assays. The sensitivity and specificity of five independent primer sets were determined on self collected vaginal specimens obtained from female commercial sex workers. Methods: Self collected specimens were obtained from symptomatic and asymptomatic women attending a female sex workers clinic in Abidjan, Cote d'Ivoire. Two vaginal specimens were collected, the first one was processed for culture and the second was processed for PCR analysis. PCR techniques for trichomonads were performed, using the primers as reported by Riley (TVA5/TVA6), Kengne (TVK3/TVK7), Madico (BTUB 9/BTUB 2), Shiao (IP1/IP2), and Mayta (TV1/TV2). An EIA amplicon detection method was designed for each of the primer sets. Results: True positive specimens were defined as culture positive and/or two positive PCR results with EIA amplicon detection in any combination. According to this definition a prevalence of 20% was obtained compared to 7% obtained by culture. The PCR primer set TVK3/TVK7 gave the highest sensitivity (89.2%). Poor sensitivities were obtained with the primer sets TV1/TV2 (60.2%) and TVA5/TVA6 (63.9%). PCR showed a sensitivity improvement of 2.4% up to 12% when EIA was used for amplicon detection. Conclusions: Overall, the sensitivities of the different PCR assays resulting from this study were lower than those previously described. These findings could be the result of the nature of the


Objectives: To determine seroprevalence and determinants of herpes simplex virus 2 (HSV-2) seropositivity, in a random sample of a population based cohort of 10,049 women of Guanacaste, Costa Rica, using a highly sensitive and specific serological assay. Methods: Seroprevalence was determined by a type specific HSV-2 ELISA assay in an age stratified random sample of 1,100 women. Univariate and multivariate logistic regression was used to calculate odds ratios and 95% confidence intervals for risk factors of seropositivity. Results: Overall age adjusted HSV-2 seroprevalence was 38.5% (95% CI, 37.5 to 39.5), and it was strongly associated with increasing age (pTrend<0.0001), both among monogamous women and women with multiple sexual partners. A greater number of lifetime sexual partners increased the risk of seropositivity, with a 28.2% (95% CI, 24.4 to 32.2) seroprevalence among monogamous women and 75% (95% CI, 65.6 to 83.0) seroprevalence for those with four or more partners (OR = 7.6 95% CI, 4.7 to 12.4 pTrend<0.0001). Barrier contraceptive use was negatively associated with HSV-2 seropositivity (OR 0.54, 95% CI, 0.31 to 0.94). Women with antibodies against HPV 16, 18, or 31 were 1.6 times more likely to be HSV-2 seropositive (OR 1.6, 95% CI, 1.2 to 2.1). Conclusions: HSV-2 infection is highly endemic in Guanacaste, even among lifetime monogamous women, suggesting a role of male behaviour in the transmission of the infection. Until vaccination against HSV-2 is available, education to prevent high risk sexual behaviour and the use of condoms appear as preventive measures against HSV-2.

Small Ruminant Research


Semen and peripheral blood mononuclear cells (PBMC) samples of 15 infected and 3 non-infected bucks were evaluated for the presence of caprine arthritis-encephalitis virus (CAEV), by double-nested polymerase chain reaction (d-n-PCR). In order to locate the presence of virus, semen samples were separated into cell-free seminal fluids (CFSF), enriched non-spermatozoal cells fraction (NSCF), and spermatozoa (SPZ). All PBMC samples from infected bucks were positive for CAEV-DNA but only 8 out of 15 semen samples were positive for CAEV-DNA. The presence of virus was not necessarily concordant in different fractions of semen from the same buck over time. In addition, the virus was isolated from CFSF in primary goat synovial membrane (GSM) cell culture, in which productive infection was demonstrated by syncytia formation. This is the first report describing the presence of CAEV-DNA and also of replication-competent virus in semen from CAEV seropositive bucks. Such results suggest the possibility of sexual transmission of CAEV.

Soil Biology and Biochemistry

Soil Biology and Biochemistry (13)

http://www.sciencedirect.com/science/article/B6TC7-46SW58F-6/2/c545c4562f9ec411e532c7211c93452f

The distribution, diversity and relative abundance of Sesbania sesban rhizobia in African soils were investigated by host-trapping and counting of rhizobia and characterization using restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA and the internally transcribed spacer (ITS) between the 16S and 23S rRNA genes. Isolates representative of the diverse 16S rRNA groups from the various soils were selected for sequence analysis of the first 750 bp of the 16S rRNA. Compatible rhizobia were detected in only 15 out of 55 soils, and were present generally in soils with more than 10% clay, and those from low-lying areas. Populations were small, generally much less than 50 cells g-1 soil. The rhizobia nodulating S. sesban were genetically diverse, with isolates bearing 16S rRNA sequences similar to those of rhizobia belonging to the genera Rhizobium, Mesorhizobium, Sinorhizobium and Allorhizobium. About 1% of the isolates recovered had sequences with close homology with *Agrobacterium tumefaciens*. Despite the wide phylogenetic distribution of the rhizobial isolates, the Mesorhizobium group was dominant in all soils examined, accounting for 90% of the isolates on average, with individual soil populations usually being comprised of two genera. There was a marked variability in the sequence and size of the ITS region among rhizobia nodulating Sesbania which indicates a broad diversity of 'strain' types both within and between soil populations, and within and between rhizobial genera.


http://www.sciencedirect.com/science/article/B6TC7-3W3NCHS-3/2/75f12c92f91eab89db03e892a3c1853d5

Our objective was to determine the effect of gut transit retention time of genetically-modified bacteria ingested by the woodlouse Porcellio scaber. The experimental animals were supplied ash leaf litter inoculated with the genetically-modified bacterium *Pseudomonas fluorescens* KTG and bacteria in food and faeces were counted using selective plating and immunofluorescent techniques. The bacteria were also detected using the polymerase chain reaction (PCR). It was found that plate counts of *P. fluorescens* KTG in fresh faeces were lower than those found in the litter when the GEMMO was supplied to animals at five different population densities, suggesting that a proportion of the GEMMO population was lost during gut transit. There was no significant difference in the survival of freshly cultured and starved cultures of *P. fluorescens* KTG on gut transit through *P. scaber* as determined by plate counts in fresh faeces. Retention time of *P. fluorescens* KTG in the woodlouse gut was found to be longer than that of the food bolus. The passage of bacteria through the gut was modelled and tracked using microbeads of a size similar to bacteria. Fluorescent microbeads added to food litter were detected within the anterior chamber, papillate region and rectum of the woodlouse for at least 17 days after ingestion. Scanning electron microscopy revealed that beads were retained within the cuticular structure of the digestive tract and also within mucopolysaccharide produced within the gut. Immunofluorescent observations of washed hindgut samples provided little evidence to suggest *P. fluorescens* KTG had become attached to the hindgut wall during transit. Very few colonies of the GEMMO and indigenous bacteria were detected from homogenised hepatopancreas samples. *P. fluorescens* KTG was however detected in the hepatopancreas of *P. scaber* using PCR. It is suggested that the retention of bacteria within the guts of woodlice is by physical rather
than biological mechanisms such as growth or attachment.


http://www.sciencedirect.com/science/article/B6TC7-42991JB-J/2/7aeb7f5426aebe6838f1c260d6d3ec56a9

The equilibrium adsorption and binding of the active toxin from Bacillus thuringiensis subsp. kurstaki on complexes of montmorillonite-humic acids-Al hydroxypolymers, as well as the biodegradation and the insecticidal activity of the bound toxin, were studied. Seventy percent of the total adsorption occurred within the first hour, and maximal adsorption occurred in 2O and 1 M NaCl. The bound toxin was resistant to utilization by mixed microbial cultures from soil and to enzymatic degradation by Pronase E. Free and bound toxin were active against the larvae of Manduca sexta; the bound toxin retained the same activity after exposure to microbes or Pronase, whereas the toxicity of the free toxin decreased significantly. The results of these studies indicate that the release of transgenic plants and microorganisms expressing truncated genes that encode active insecticidal toxins from B. thuringiensis could result in the accumulation of these toxins in soil as a consequence of binding on surface-active soil particles. This persistence could pose a hazard to nontarget organisms, enhance the selection of toxin-resistant target species, and increase the control of target insect pests.


http://www.sciencedirect.com/science/article/B6TC7-4CY5JPN-3/2/d9ebc3d1f705454d19afbf1c4d207e98

The impact of DNA extraction protocol on soil DNA yield and bacterial community composition was evaluated. Three different procedures to physically disrupt cells were compared: sonication, grinding-freezing-thawing, and bead beating. The three protocols were applied to three different topsoils. For all soils, we found that each DNA extraction method resulted in unique community patterns as measured by denaturing gradient gel electrophoresis. This indicates the importance of the DNA extraction protocol on data for evaluating soil bacterial diversity. Consistently, the bead-beating procedure gave rise to the highest number of DNA bands, indicating the highest number of bacterial species. Supplementing the bead-beating procedure with additional cell-rupture steps generally did not change the bacterial community profile. The same consistency was not observed when evaluating the efficiency of the different methods on soil DNA yield. This parameter depended on soil type. The DNA size was of highest molecular weight with the sonication and grinding-freezing-thawing procedures (approx. 20 kb). In contrast, the inclusion of bead beating resulted in more sheared DNA (approx. 6–20 kb), and the longer the bead-beating time, the higher the fraction of low-molecular weight DNA. Clearly, the choice of DNA extraction protocol depends on soil type. We found, however, that for the analysis of indigenous soil bacterial communities the bead-beating procedure was appropriate because it is fast, reproducible, and gives very pure DNA of relatively high molecular weight. And very importantly, with this protocol the highest soil bacterial diversity was obtained. We believe that the choice of DNA extraction protocol will influence not only the determined phylogenetic diversity of indigenous microbial communities, but also the obtained functional diversity. This means that the detected presence of a functional gene—and thus the indication of enzyme activity—may depend on the nature of the applied DNA extraction procedure.

http://www.sciencedirect.com/science/article/B6TC7-473M734-5/2/49412a0c40c9950d801d5af2fbbfe1187

When assessing bacterial community structure in soil it is important to establish a satisfactory procedure for sampling. The influence of sample sizes of a forest soil on the assessment of bacterial community structure was investigated. Four sample sizes (0.01, 0.1, 1.0, and 10.0 g) were evaluated. Time of colony appearance on a nutrient-limited soil extract agar was used to characterise the culturable heterotrophic and Pseudomonas communities. Genetic community structure was assessed with denaturing gradient gel electrophoresis (DGGE) of 16S rDNA using a bacterial primer set. The largest variation in the heterotrophic community structure for bacteria was seen when comparing the 0.01 g replicates. Variation was also seen for the 0.1 and 1.0 g replicates. However, there was no significant difference between the 10.0 g replicates. The 0.01 and 0.1 g replicates showed variation in genetic community structure within the sample sizes, whereas variation between the replicates within the larger sample sizes (1.0, 10.0 g) was negligible. Variation in the heterotrophic community structure for Pseudomonas was seen between replicates of all sample sizes. Hence, the size of soil samples influenced the bacterial community structure observed for bacteria, whereas chance seemed to play an important role when looking at a more narrow community structure.


http://www.sciencedirect.com/science/article/B6TC7-4C1CBRK-6/2/359b1ed9173ce84cb5c1433b40362f6a

As part of the restoration of biodiversity on former agricultural land there has been focused on methods to enhance the rate of transition from agricultural land towards natural grasslands or forest ecosystems. Management practices such as sowing seed mixtures and inoculating soil of later successional stages have been used. The aim of this study was to determine the effects of a managed plant community on the diversity of soil fungi in a newly abandoned agricultural land. A field site was set up consisting of 20 plots where the plant diversity was managed by either sowing 15 plant species, or natural colonization was allowed to occur. The plant mixture contained five species each of grasses, legumes and forbs that all were expected to occur at the site. A subset of the plots (five from each treatment) was inoculated with soil cores from a late successional stage. The plant community composition was subject to a principal component analysis based on the coverage of each species. Five years after abandonment, soil samples were taken from the plots, DNA was extracted and the ITS region of the rDNA gene was amplified using fluorescently labelled fungal specific primers (ITS 1F/ITS 4). The PCR products were digested using Hinfl and TaqI and sequenced. Results from both restriction enzymes were combined and a principal component analysis performed on the presence/absence of fragments. Also the fungal diversity expressed as number of restriction fragments were analysed. There was significantly higher fungal species richness in the experimental plots compared to the forest and field soils, but no differences between sown and naturally colonized plots. The different plant treatments did not influence the below ground fungal community composition. Soil water content on the other hand had an impact on the fungal community composition.

http://www.sciencedirect.com/science/article/B6TC7-40PGT9H-B/2/6cdc1b8f118cc2d1e3fc0f1d65923946

We have used PCR based on 16S rDNA sequences followed by denaturing gradient gel electrophoresis (PCR-DGGE) in conjunction with cultivation-based methods to describe the effect of artificial root exudates (ARE), of which the composition simulated maize root exudates, on the structural diversity of bacterial communities in various soils differing in the level of contamination with heavy metals. The aim of this study was to evaluate the effects of organic compounds of a root exudates as a potential mechanism for selectively enhancing specific bacterial populations in contaminated soils, leading to the development of shifted communities differing in qualitative and quantitative composition. Soil microcosms were either just enriched with ARE or enriched and, additionally, flooded. To characterise the response of the soil microflora to the enrichment, PCR-DGGE was applied for assessment of the total bacterial community structure. Cultivation techniques were used to determine the numbers of total heterotrophic bacteria as well as of pseudomonads (which are considered to be stimulated by components of root exudates). The community structure of culturable bacteria was studied using the concept of r- and K-strategists, and isolates from dominant colonies growing on King's B agar were identified by MIDI-FAME profiling. The results obtained showed a significant effect of root exudates on the development of bacterial populations in soil contaminated with heavy metals. Depending on their availability and conditions prevailing in the habitat (e.g. stronger enrichment by flooding) different bacterial populations were stimulated, resulting in generation of different community patterns by DGGE. The most significant response to root exudates occurred among the culturable fraction of the soil bacteria. Distribution of bacterial classes (i.e. majority of colonies appeared after 24 h), values of EP (from 0.220 to 0.533) and CD (from 43 to 88) indices directly showed that the culturable fraction of bacteria was highly affected by the organic mixture simulating root exudates. These exudates reduced the bacterial diversity towards domination of r-strategists and the reduction of diversity was greater in soil with a higher contamination level. Furthermore, flooding of the soils enhanced the dominance of fast growing bacteria (over 70% formed visible colonies after 24 h even on day 6) and reduced the community diversity (EP and CD indices were from about 0.291 to 0.425 and from 66 to 87, respectively).


http://www.sciencedirect.com/science/article/B6TC7-485X6R1-1/2/182e687dcc2b629321e1ff5d1c05776c

Seven isolates of Rhizobium leguminosarum bv. viciae (Rlv) that nodulate faba beans (Vicia faba) from six sites in Jordan were characterised for chromosomal (glnII) and symbiotic (nodD-F) genotypes using polymerase chain reaction-restriction fragment length polymorphism and sequencing methods. The results were compared to those obtained in a previous UK study, to determine whether or not the UK field population are indigenous or if they were dispersed during the radiation of V. faba domestication. All seven Jordanian isolates displayed novel chromosomal and symbiotic genotypes not identified in the UK population.

The polymerase chain reaction (PCR) amplification of specific DNA sequences, allows specific and sensitive detection of bacteria at the genus, species or strain level depending on the design of the oligonucleotide primers. In this study we utilized 20 mer primers that flanked a 300 bp region of the npt II gene of the transposon Tn5 thus allowing for the amplification of this region. Insertion of the Tn 5 element into rhizobia allowed for detection of these cells using PCR amplification. Using the npt II-specific primers and Tn5 insertion mutants of Rhizobium leguminosarum bv. phaseoli we were able to detect these specific rhizobia strains in root nodules of bean plants and in inoculated soils. Utilization of genus-specific gene sequences would allow for estimates of cells of that genus in environmental samples. Conversely, use of gene sequences common to rhizobia, e.g. nif and nod sequences, would give estimates of the population of rhizobia. This paper serves to illustrate the use of PCR, for detecting gene sequences in an environmental sample such as a root nodule.


Heavy metal availability, microbial biomass and respiration, bacterial diversity and enzyme activity were studied in soils from long-term field experiments contaminated with Mn-Zn- or Cd-Ni-rich sludge, incorporated into soils at two different rates. Soils that never received sludge were used as controls. Microbial biomass C content (BC) and soil respiration (CO2-C) were slightly reduced in soils amended with Mn-Zn at the higher incorporation rate whereas in soils receiving Cd-Ni-rich sludge BC and respiration were unaffected. Metabolic quotient values (qCO2) calculated by the BC-to-CO2-C ratio were not significantly different, regardless of the sludge type whereas the microbial biomass C-to-total organic C (BC-to-TOC) ratios were significantly reduced in the soils receiving the higher rates of both sludge types. Phosphomonoesterase, [beta]-glucosidase and arylsulfatase activities and hydrolase-to-BC ratios, were significantly reduced in soils amended with Ni-Cd-sludge at both rates, whereas the Mn-Zn-sludge only reduced the arylsulfatase activity at the higher rate. Protease activity was generally higher in all the sludge-amended soils as compared to control soils whereas urease activity was unaffected by sludge amendments. The structure of the bacterial community, as determined by denaturing gradient gel electrophoresis (DGGE), was different in the sludge-amended soils as compared to the respective controls. The most important changes were observed in the soils amended with high-level Ni-Cd sludge. Because some of the adverse effects were observed at moderate contamination levels, our results indicate that the presence of certain heavy metal combinations can be a serious limitation for sludge disposal.


We studied the effect of humified organic matter (OM) on the mineralization of a representative labile organic compound in soil. In an incubation experiment, a 13C-labeled 2-decanol was added to soil either alone (2-dec*) or in mixture with two humic acids from compost (HAC*) and lignite (HAL*) which had different hydrophobic properties. Isotopic dilution ([delta]13C) showed that after 3 months of incubation about 58, 40 and 28% of the added 13C was retained in the whole soil.
treated with HAL*, HAC* and 2-dec*, respectively. The higher the hydrophobicity of the employed humic material, the larger was the sequestration of organic carbon in soil. Fractionation of incubated samples revealed that the labeled carbon progressively accumulated in the finest particle-size fractions. However, the high hydrophobicity of the lignite HA favored accumulation of 13C also in the sand-sized fraction. The NMR spectra of humic extracts showed that the 13C-methyl group in the original 2-decanol had been oxidized to a 13C-carboxyl group during incubation for all treatments. This indicated that despite its hydrophilicity, the resulting carboxyl carbon was sequestered into the hydrophobic domains of the humic pool in soil. In fact, the residual 13C was larger in humic than in fulvic extracts for the control sample (2-dec*) and even more so in extracts from soil treated with both exogenous humic acids. Our results suggest that labile organic compounds may be effectively protected in soil by humified OM and their microbial mineralization substantially reduced. Innovative soil management practices employing hydrophobic humic substances may increase the biological stability of soil OM and thus contribute to significantly mitigate CO2 emissions from agricultural soils.


http://www.sciencedirect.com/science/article/B6TC7-3YF4MX8-77/2/982db2f637c0a2a7db9b27eddf905ae

A simple method for the detection of small populations of Pseudomonas fluorescens P.B8-1, containing the nptII gene of Tn5 as a unique marker, was applied to a Nyuzen paddy soil using cell extraction (indirect DNA extraction) and a "nested" polymerase chain reaction (PCR). This involved processing samples through a combination of a sucrose gradient centrifugation procedure to isolate bacterial cells, followed by cell lysis with proteinase K and CTAB (hexadecyltrimethyl ammonium bromide)-NaCl. This method allowed the extraction of DNA within about 6 h followed by amplification of DNA. The optimized "nested" PCR comprised a "2-step" PCR (45 cycles) using two 20-mer primers, followed by a "3-step" PCR (30 cycles) using two 26-mer primers which were internal to the first set. After the first PCR step was performed, the amplified DNA was detectable from the inoculated soil containing a minimum of 105 cfu g-1. However, the "nested" PCR procedure permitted the detection of amplified DNA fragments from inoculated non-sterile soils containing 1.3 x 101 cfu g-1. The application of this detection strategy was tested by monitoring the survival of P. fluorescens P.B8-1 in a non-sterile paddy soil during a 53-day period. The P.B8-1 population decreased in soils maintained at either 25 or 10[deg]C after inoculation. After 53 days, samples of soil maintained at 10[deg]C contained 102 cfu g-1 of P.B8-1 (as determined by selective plate count) and permitted amplification of DNA by the "nested" PCR. At the same time, P.B8-1 was not detected in soil maintained at 25[deg]C by either method. The results obtained using this detection strategy suggest that it is highly applicable to monitoring the fate of genetically engineered microorganisms in natural paddy soils.


http://www.sciencedirect.com/science/article/B6TC7-3VXYG8T-D/2/06f31ea4f181016838c1a71dd6f43f95

Adsorption at equilibrium and binding of AmpliTaq(R) DNA polymerase (TDP) on clay minerals, two montmorillonites (W-M and Ap-M) and a kaolinite (K), were studied. Equilibrium adsorption isotherms were of the L type, and a plateau was reached with Ap-M and K, whereas no plateau was obtained with W-M. Adsorption of TDP was most rapid on W-M, maximal at pH 6 on all clays,
and appeared to involve hydrophobic interactions between the clays and TDP. The amounts of TDP adsorbed and bound on the clays were higher on W-M than on Ap-M and K and occurred only on the external surfaces of the clays. Fourier-transform infrared spectra, SDS-PAGE and scanning electron microscopy of both pure TDP and the W-M-TDP complex showed the presence in the commercial TDP of microspheres (about 100 nm in diameter) of unidentified material, which could influence the adsorption-binding of TDP on clays.

**Stem Cells** (6)


http://stemcells.alphamedpress.org/cgi/content/abstract/22/4/600

Earlier studies reported that neural stem (NS) cells injected into blastocysts appeared to be pluripotent, differentiating into cells of all three germ layers. In this study, we followed in vitro green fluorescent protein (GFP)-labeled NS and embryonic stem (ES) cells injected into blastocysts. Forty-eight hours after injection, significantly fewer blastocysts contained GFP-NS cells than GFP-ES cells. By 96 hours, very few GFP-NS cells remained in blastocysts compared with ES cells. Moreover, 48 hours after injection, GFP-NS cells in blastocysts extended long cellular processes, ceased expressing the NS cell marker nestin, and instead expressed the astrocytic marker glial fibrillary acidic protein. GFP-ES cells in blastocysts remained morphologically undifferentiated, continuing to express the pluripotent marker stage-specific embryonic antigen-1. Selecting cells from the NS cell population that preferentially formed neurospheres for injection into blastocysts resulted in identical results. Consistent with this in vitro behavior, none of almost 80 mice resulting from NS cell-injected blastocysts replaced into recipient mothers were chimeric. These results strongly support the idea that NS cells cannot participate in chimera formation because of their rapid differentiation into glia-like cells. Thus, these results raise doubts concerning the pluripotency properties of NS cells.


http://stemcells.alphamedpress.org/cgi/content/abstract/22/2/225

The transcription factor Oct-4 is a marker of pluripotency in mouse and human embryonic stem (ES) cells. Previous studies using a tetracycline-regulated Oct-4 transgene in the ZHBTc4 cell line demonstrated that downregulation of Oct-4 expression induced dedifferentiation into trophoblast, a lineage mouse ES cells do not normally generate. We found that transfection of Oct-4-specific short interfering RNA significantly reduced expression and functional activity of Oct-4 in mouse and human ES cells, enabling its role to be compared in both cell types. In mouse ES cells, Oct-4 knockdown produced a pattern of morphological differentiation and increase in expression of the trophoblast-associated transcription factor Cdx2, similar to that triggered by suppressing the Oct-4 transgene in the ZHBTc4 cell line. In addition, downregulation of Oct-4 was accompanied by increased expression of the endoderm-associated genes Gata6 and (alpha)-fetoprotein, and a gene trap associated with primitive liver/yolk sac differentiation. In human ES...
cells, Oct-4 knockdown also induced morphological differentiation coincident with the upregulation of Gata6. The induction of Cdx2 and other trophoblast-associated genes, however, was dependent on the culture conditions. These results establish the general requirement for Oct-4 in maintaining pluripotency in ES cells. Moreover, the upregulation of endoderm-associated markers in both mouse and human ES cells points to overlap between development of trophoblast and endoderm differentiation.


http://stemcells.alphamedpress.org/cgi/content/abstract/20/5/402

Previously, we investigated the process of megakaryocytopoiesis during ex vivo expansion of human cord blood (CB) CD34+ cells using thrombopoietin (TPO) and found that megakaryocytopoiesis was closely associated with apoptosis. To understand megakaryocytopoiesis at the molecular level, we performed a microserial analysis of gene expression (microSAGE) in megakaryocytes (MKs) and nonmegakaryocytes (non-MKs) derived from human CB CD34+ cells by ex vivo expansion using TPO, and a total of 38,909 tags, representing 8,976 unique genes, were identified. In MKs, many of the known genes, including coagulation factor VII, P-selectin (CD62P), pim-1, azurocidin, defensin, and CD48 were highly expressed; meanwhile, those genes encoding some small G proteins of the Ras family (Rab 7 and Rab 11A) and glutathione S transferase family (1, 4, A2, omega, and pi) showed lower expression levels in MKs. These gene expression profiles will be useful to understand megakaryocytopoiesis at the molecular level, including apoptosis and related signal transduction pathways.


http://stemcells.alphamedpress.org/cgi/content/abstract/21/6/681

The in vitro culture of human trabecular bone-derived cells has served as a useful system for the investigation of the biology of osteoblasts. The recent discovery in our laboratory of the multilineage mesenchymal differentiation potential of cells derived from collagenase-treated human trabecular bone fragments has prompted further interest in view of the potential application of mesenchymal progenitor cells (MPCs) in the repair and regeneration of tissue damaged by disease or trauma. Similar to human MPCs derived from bone marrow, a clearer understanding of the variability associated with obtaining these bone-derived cells is required in order to optimize the design and execution of applicable studies. In this study, we have identified the presence of a CD73+, STRO-1+, CD105+, CD34-, CD45-, CD144- cell population resident within collagenase-treated, culture-processed bone fragments, which upon migration established a homogeneous population of MPCs. Additionally, we have introduced a system of culturing these MPCs that best supports and maintains their optimal differentiation potential during long-term culture expansion. When cultured as described, the trabecular bone-derived cells display stem cell-like capabilities, characterized by a stable undifferentiated phenotype as well as the ability to proliferate extensively while retaining the potential to differentiate along the osteoblastic, adipocytic, and chondrocytic lineages, even when maintained in long-term in vitro culture.


http://stemcells.alphamedpress.org/cgi/content/abstract/23/3/315

Previous studies have shown that prolonged propagation of undifferentiated human embryonic stem cells (hESCs) requires conditioned medium from mouse embryonic feeders (MEF-CM) as well as matrix components. Because hESCs express growth factor receptors, including those for basic fibroblast growth factor (bFGF), stem cell factor (SCF), and fetal liver tyrosine kinase-3 ligand (Flt3L), we evaluated these and other growth factors for their ability to maintain undifferentiated hESCs in the absence of conditioned medium. We found cultures maintained in bFGF alone or in combination with other factors showed characteristics similar to MEF-CM control cultures, including morphology, surface marker and transcription factor expression, telomerase activity, differentiation, and karyotypic stability. In contrast, cells in media containing Flt-3L, thrombopoietin, and SCF, individually or in combination, showed almost complete differentiation after 6 weeks in culture. These data demonstrate that hESCs can be maintained in nonconditioned medium using growth factors.


http://stemcells.alphamedpress.org/cgi/content/abstract/23/5/610

Recent studies have indicated that bone marrow cells can regenerate damaged muscles and that they can adopt phenotypes of other cells by cell fusion. Our direct visualization system gave evidence of massive muscle regeneration by green fluorescent protein (GFP)-labeled CD45+c-Kit+Sca-1+Lin- cells (KSL cells), and we investigated the role of KSL cells in muscle regeneration after transplantation with or without lethal irradiation. In the early phase, GFP signals were clearly observed in all the muscles of only irradiated mice. Transverse cryostat sections showed GFP+ myosin+ muscle fibers, along with numerous GFP+ hematopoietic cells in damaged muscle. These phenomena were temporary, and GFP signals had dramatically reduced 30 days after transplantation. After 6 months, GFP+ fibers could hardly be detected, but GFP+ c-Met+ mononuclear cells were located beneath the basal lamina where satellite cells usually exist in both conditioned mice. Immunostaining of isolated single fibers revealed GFP+ PAX7+, GFP+ MyoD+, and GFP+ Myf5+ satellite-like cells on the fibers. Single-fiber cultures from these mice showed proliferation of GFP+ fibers. These results indicate two different roles of KSL cells: one leading to regeneration of damaged muscles in the early phase and the other to conversion into satellite cells in the late phase.

Steroids (11)


http://www.sciencedirect.com/science/article/B6TC9-408BJK1-3/2/e6d52b0b231ccce7abab3d2b7f4d85e3
Using reverse transcriptase polymerase chain reaction (RT-PCR) with degenerate primers followed by 3’ rapid amplification of cDNA ends PCR (3’Race-PCR) we have isolated a new fish steroid receptor cDNA sequence of 1806 bp from rainbow trout (Oncorhynchus mykiss) testis. This sequence has clear homology with various mineralocorticoid receptor cDNA sequences (rat, human, African toad: 68-70% amino acid identity), and encompasses the second part of DNA binding domain (C domain), the whole hinge region (D domain) and the steroid binding domain (E domain) plus 726 bp of 3’untranslated sequence. COS-1 cells transfected with a pCMV5 expression vector containing the whole E domain (pCMV5-rtMR) showed high affinity binding for cortisol (Ka = 0.53 +/- 0.03 nM, Kd = 1.9 nM) in the cytosol, which could not be detected in untransfected cells. Aldosterone displaced 3H-cortisol binding, though was less effective than unlabeled cortisol (P 3H-cortisol from the receptor-ligand complex: cortisol = corticosterone = 11-deoxycortisol = 21-deoxycortisol > 11-deoxycorticosterone = 11[beta]-hydroxyprogesterone = 17-hydroxyprogesterone > dexamethasone, whereas 17,20[beta]-dihydroxy-4-pregnen-3-one and 17,20[beta],21[beta]-trihydroxy-4 pregnen-3-one (two fish specific progestins) did not show any specific binding. These results strongly suggest that this cDNA sequence encodes a rainbow trout mineralocorticoid-like receptor, and represent the first description of such a receptor in teleost fish where aldosterone, the classic mineralocorticoid, is believed to be absent.


http://www.sciencedirect.com/science/article/B6TC9-40PRGST-2/2/e05b558424e4ecfeeed9a40bf577eef55

In ovariectomized (Ovx) mice, collagenolytic cysteine protease (CCP) activity in calvaria significantly increased 7 days after ovariectomy and was about 50% of that observed in sham-operated (Sham) mice 3 weeks later. In Ovx mice, subcutaneously (s.c.) administered estradiol-17[beta] (E2) (10 [mu]g/kg) for 2 weeks led to a decrease in CCP activity in calvaria to the level observed in Sham mice. In Ovx mice, though the amount of cathepsin L increased more than that of cathepsin K, cathepsin K and cathepsin L content increased by 200-400% compared with the Sham mice; cathepsin K was detected in larger amounts than cathepsin L in calvaria from both Sham and Ovx mice. The amounts of cathepsin K and cathepsin L in Ovx mice were reduced to the values seen with Sham mice after administration (s.c.) of E2 (10 [mu]g/kg) for 2 weeks. In mouse calvarial organ culture, the increase of CCP activity and release of hydroxyproline, an indicator of degradation of type-I collagen, in the presence of 1[alpha],25-(OH)2D3, parathyroid hormone, interleukin (IL)-1[alpha], IL-6, or tumor necrosis factor-[alpha] was suppressed by E2 (10-9-10-7 M). In all cases, secretion of both cathepsin K and cathepsin L were suppressed by E2. In osteoclasts, expression of cathepsin K and cathepsin L was suppressed by E2 at the mRNA level. Cathepsin B was detected faintly or not at all. These results suggest that synthesis of cathepsin K and cathepsin L was negatively regulated by E2 at the mRNA level. In Ovx mice, deficiency of E2 resulted in an augmentation of cathepsin K and cathepsin L synthesis, and the cathepsins might share roles in bone resorption in vivo.


http://www.sciencedirect.com/science/article/B6TC9-4FM01J9-1/2/e34eda6b080db206ec6aaa0a5c92d9af

A subset of lipophillic bile acids, including deoxycholic acid (DCA) and lithocholic acid (LCA), are thought to be biologically transformed into reactive intermediates forming covalently modified, "tissue-bound" bile acids that can exert several toxic effects. We have generated a single-chain
Fv fragment (scFv) as a probe to monitor DCA residues anchored on proteins. DNA fragments encoding the variable heavy (VH) and light (VL) domains of a mouse antibody raised against a DCA hapten (Ab #88) were cloned by rapid amplification of cDNA 5'-ends. These sequences were combined via a common linker sequence coding (Gly4Ser)3 to construct a single scFv gene with the gene segments in the following order: 5'-VH-linker-VL-3'. This construct was subcloned into an antibody-expression vector, pEXmide 5; soluble scFv protein was then expressed in the bacterial periplasm of the XLOLR Escherichia coli strain. In a competitive enzyme-linked immunosorbent assay using DCA-coated microtiter plates, the scFv provided a dose-response curve for free DCA ranging between 2 and 5000 pg/assay. The scFv reacts similarly with the L-lysine adduct of DCA (cross-reactivity, 72%), while bile acids having a modified DCA steroid skeleton were well-discriminated (cross-reactivity, <1%). This scFv could also monitor trace amounts of DCA residues anchored on a protein through DCA acyl adenylate reactions, the likely reactive intermediate. The present scFv may be a useful tool for trace characterization of tissue-bound bile acids; this usefulness may be significantly enhanced by fusion with signal-generating proteins, such as alkaline phosphatase or green fluorescent protein.


http://www.sciencedirect.com/science/article/B6TC9-45V71TJ-1/2/ab91ec8ff35dc6c7b90a23957325a3a0

Single-chain Fv fragments (scFvs) against a corticosteroid, 11-deoxycortisol (11-DC), have been generated as a template antibody fragment from which a comprehensive mutated antibody library containing various anti-steroid antibodies could be constructed. The cDNAs encoding variable heavy (VH) and light (VL) domains of a mouse anti-11-DC antibody (CET-M8), were amplified by RT-PCR, combined via a common linker to construct the sequence of 5'-VH-(Gly4Ser)3-VL-3', and cloned into a phagemid vector, pEXmide 5. The phage clones exhibiting binding activity to 11-DC were isolated after single panning against a hapten-immobilizing immunotube. The scFv gene in one of these clones was reamplified to introduce the ochre codons, and then expressed in the bacterial periplasm as the soluble antibody fragment. Two different scFvs (#6 and #12) were cloned, whose binding characteristics were examined by a radioimmunoassay using a tritium-labeled 11-DC. Both of them showed high affinity (Ka=1.3 x 1010 M-1) and practical specificity (cross-reactivity: cortisol, <0.2%; cortisone, <0.3%) to 11-DC, and furthermore, strong reactivity with an anti-idiotype antibody which recognizes the paratope of CET-M8. These results suggest that the present scFvs retain the three-dimensional structure of the paratope of the original monoclonal antibody.


http://www.sciencedirect.com/science/article/B6TC9-3YYV2WJ-1S/2/6857e3b1077c23d9bb8eefcc904f3c32

This study examined estrogen receptor dynamics in the livers of male obeses rats (SHHF/Mcc-cp) treated for two weeks with a continuous, low dose of 17[beta]-estradiol compared with untreated controls. An increased binding capacity for tritiated 17[beta]-estradiol in the cytosol, consistent with binding to the estrogen receptor, was demonstrated in treated males relative to control males (P < 0.01). These observations were confirmed using curve-peeling techniques with saturation analysis, ammonium sulfate precipitation/fractionation of cytosol protein, and chromatographic techniques to isolate the high-affinity binding from other interfering factors. Increased hepatic nuclear estrogen receptor levels in treated males (112.3 +/- 8.3 fmol/g liver) compared with
controls (64.1 +/- 6.8 fmol/g liver) suggested that the liver was under estrogenic influences. This interpretation was supported by an increase in serum triglyceride levels, reflecting increased very low density lipoprotein secretion by the liver. Reductions in testosterone levels and in the weights of seminal vesicles and the testes in treated males indicated detrimental effects on reproduction. An interpretation of increased synthesis of estrogen receptor with 17[beta]-estradiol treatment was supported by the observation of an increase in the mRNA for estrogen receptor. Taken together, these observations indicate that continuous, low-dose 17[beta]-estradiol treatment induces estrogenic action in the livers of male rats and also increases hepatic estrogen receptor, probably indirectly, via an increase in its mRNA.


http://www.sciencedirect.com/science/article/B6TC9-47NVJXY-BX/2/f5d1e6c20bb05d412cd9a9e9f22600b6

Previous studies have shown that the gonadotropins follicle-stimulating hormone and luteinizing hormone stimulate proopiomelanocortin (POMC) promoter activity and mRNA levels in ovarian granulosa cells. The objective of these studies was to determine the role of cAMP-dependent protein kinases (pKA) in gonadotropin-stimulated gene expression. Primary cultures of rat granulosa cells were transfected with a gene construct consisting of the POMC promoter (-150 to + 63; designated pOMC-CAT) fused to the chloramphenicol acetyltransferase (CAT) reporter gene either alone or cotransfected with an expression plasmid (designated mutant RI), which overexpresses a mutant form of the murine RI subunit incapable of binding cAMP and serving as an irreversible inhibitor of the catalytic subunit of pKA. Follicle-stimulating hormone or isoproterenol caused a significant stimulation of pOMC-CAT activity in transfected cells. Cotransfection of pOMC-CAT with mutant RI caused a significant inhibition of basal pOMC-CAT activity and abolished the gonadotropin stimulation. As a control, transfection of the SV-40 viral enhancer-promoter fused to CAT (pSV2-CAT) was unresponsive to follicle-stimulating hormone stimulation and cotransfection with mutant RI had no significant effect on pSV2-CAT activity. These studies suggest that gonadotropin regulation of the POMC promoter is mediated by pKA and that promoter activity is stringently controlled by pKA.


http://www.sciencedirect.com/science/article/B6TC9-47P835S-51/2/21f67397471816a510306938c857d03f

Corticosteroid-binding globulin (CBG or transcortin) is a specific plasma glycoprotein, which binds steroid hormones (cortisol, corticosterone, and progesterone), and plays a role in transporting these steroids, altering their concentrations in blood, and influencing their biological actions. CBG has been previously shown to be synthesized in the liver, but recently it has been reported that immunoreactive CBG is localized in target tissues. In the present work, CBG mRNA was detected in normal human endometrial tissues by Northern blot analysis and reverse transcription-polymerase chain reaction. Its level was higher (P P P 59:603-607, 1994)

Since it has been demonstrated that corticosteroid-binding globulin (CBG) plays a role in intracellular steroidal actions in target cells, the expression of CBG mRNA as the measure of CBG expression was investigated in human endometrial cancers in order to assess the biological implications of CBG. The level of CBG mRNA was analyzed using competitive reverse transcription-polymerase chain reaction-Southern blot analysis. While the level of CBG mRNA was significantly (P < 0.01) higher in secretory phase endometrium than in early and late proliferative phase endometrium, the level of CBG mRNA tended to decrease with advanced dedifferentiation of endometrial cancers as compared to normal endometrium. These results suggest that dedifferentiation of endometrial cancers induces a reduction in intracellular CBG synthesis.


1α,25-(OH)2-Vitamin D3, the physiologically active metabolite of Vitamin D is known for its pro-differentiating and antiproliferative activity on various cancer cell lines. It exerts its growth-regulatory effects through binding to the Vitamin D receptor (VDR), a member of the steroid/thyroid/retinoic acid receptor family, which functions as a ligand-dependent transcription factor. There is accumulating evidence that Vitamin D may be an important determinant of both the occurrence and progression of breast cancer. Since radiation is an important etiological factor for breast cancer progression, it is important to study the role of VDR gene in radiation-induced breast carcinogenesis. This study is focused on a human breast tumor model developed by irradiating the spontaneously immortalized MCF-10F cell line with graded doses of high-linear energy transfer (LET) radiation followed by treatment with estrogen. Study of VDR gene by restriction digestion with Apal, Bsml and TaqI detected no polymorphism but direct sequencing analyses identified few single-base mutations within intron 8 and exon 9 of the gene. Over-expression of the VDR gene was noticed in irradiated and tumorigenic cell lines compared with control. Likewise, immunohistochemical data indicated a significant increase in VDR intensity in irradiated and tumorigenic cell lines. Considering all these evidence, it is likely that VDR can be used as a prognostic marker of tumor progression in radiation- and estrogen-induced breast carcinogenesis.


An association between a gene polymorphism of the human glucocorticoid receptor (hGR) gene and rheumatoid arthritis has recently been suggested. This polymorphism contains an A to G mutation in the 3'UTR of exon 9[beta], which encodes the 3'UTR of the mRNA of the hGR[beta] isoform. The hGR[beta] isoform can act as a dominant negative inhibitor of hGR[alpha], and therefore may contribute to glucocorticoid resistance. The A to G mutation is located in an AUUUA motif, which is known to destabilize mRNA. In the present study, the importance of the
mutation in this AUUUA motif was further characterized and mutations in other AUUUA motifs in the 3'UTR of hGR[beta] and hGR[alpha] mRNA were studied. hGR[beta] and hGR[alpha] expression vectors, carrying mutations in one AUUUA motif or all AUUUA motifs were transiently transfectected into COS-1 cells. Each transfected vector was analyzed for the mRNA expression level, the mRNA turnover rate and the protein expression level. The naturally occurring mutation in the 3'UTR of hGR[beta] mRNA increased mRNA stability and protein expression. Mutation of two other AUUUA motifs in the 3'UTR of hGR[beta], or mutation of all four AUUUA motifs resulted in a similar effect. Mutation of the most 5' AUUUA motif did not alter hGR[beta] mRNA expression or mRNA stability. Mutation of all 10 AUUUA motifs in the 3'UTR of hGR[alpha] mRNA increased hGR[alpha] mRNA expression and mRNA stability as well as expression of the receptor protein level. Thus, the naturally occurring mutation in an AUUUA motif in the 3'UTR of hGR[beta] mRNA results not only in increased mRNA stability, but also in increased receptor protein expression, which may contribute to glucocorticoid resistance. A similar role is suggested for two other AUUUA motifs in the 3'UTR of hGR[beta] mRNA and for the 10 AUUUA motifs that are present in the 3'UTR of hGR[alpha].


http://www.sciencedirect.com/science/article/B6TC9-48NC76Y-1/2/14bdc013c82f5757d99968db529522cb

It is known that the stress hyporesponsive period (SHRP), which seems to be related to an immature hypothalamo-pituitary-adrenal (HPA) regulatory system, occurs during the first 2 weeks after birth in rats. In the present study, we investigated the effects of sex-steroid hormones on adrenocortical responsiveness to adrenocorticotropic hormone (ACTH) in neonatal rats. The levels of cyclic adenosine 3',5'-monophosphate (cAMP), corticosterone, and adenylate cyclase activity increased with the dose of ACTH in adrenal cells of males and females in vitro. The ACTH responsiveness in adrenal cells increased with age (7-35 days of age), that is, the loss in responsiveness to ACTH just after birth began to recover in 14-35-day-old rats, but the responsiveness in 14-day-old male rats was attenuated in males compared with females. Although castration markedly augmented the responsiveness in male rats, testosterone-replacement in the castrated male rats inhibited the enhancement. Furthermore, the responsiveness in 14-day-intact female rats was suppressed by treatment with testosterone. Expression levels of ACTH receptor mRNA in adrenals increased with age in the female rat, but not in the male. Castration enhanced the level of ACTH receptor mRNA to three-fold of that in intact male rats at 14 days of age, but replacement treatment with testosterone in castrated male rats lowered the elevated levels. Testicular androgens are thought to evoke a gender-specific response in neonates, and the temporal decrease of adrenal ACTH-responsiveness might be due to the topically immature adrenal system as well as the central nervous system in mammals.

Stroke (7)


http://stroke.ahajournals.org/cgi/content/abstract/34/5/1207
Background and Purpose-- The occurrence of intracranial aneurysms and of aneurysmal subarachnoid hemorrhage are influenced by genetic factors. Recent genomic studies in Japan have defined 3 chromosomal loci and 1 haplotype of elastin polymorphisms as important risk factors, both for affected sib pairs and sporadic patients. Methods-- We have genotyped 2 single nucleotide polymorphisms in the elastin gene and evaluated their allelic association with intracranial aneurysm in a Central European sample of 30 familial and 175 sporadic patients and 235 population controls. Results-- We found no allelic association between this elastin polymorphism haplotype and intracranial aneurysm. Conclusion-- Our data probably reflect increased genetic heterogeneity of intracranial aneurysm in Europe compared with Japan.


http://stroke.ahajournals.org/cgi/content/abstract/34/11/2555

Background and Purpose-- Anecdotal evidence exists for at least 2 subpopulations of intracranial saccular aneurysms, namely, those that may form rapidly and rupture when small versus those that enlarge slowly and may rupture particularly when >10 mm in diameter. We sought to determine whether the endothelial nitric oxide synthase (eNOS) T-786C single nucleotide polymorphism (SNP), implicated in cardiovascular disease susceptibility, could facilitate differentiation between small (<=5 mm) versus large (>10 mm) ruptured aneurysms. Methods-- In accordance with institutional guidelines, clinical data were recorded prospectively and genomic DNA was isolated from blood samples obtained from 52 aneurysmal subarachnoid hemorrhage (SAH) patients (cases) and 90 randomly selected controls. Samples were assayed for eNOS gene promoter T-786C SNP with the use of gene microarray technology. Statistical analyses included multiple logistic regression. Results-- Although there was no difference in genotype distributions between cases and controls, all 13 patients with large aneurysms were (T/C) heterozygous for the polymorphism, while 9 of 22 patients (41%) with small aneurysms were (T/T or C/C) homozygous (P=0.01). The mean (+/-SD) ruptured aneurysm diameter among all heterozygotes (8.5+/-5.2 mm) was significantly greater than that for C/C (6.0+/-2.3 mm) or T/T (4.7+/-1.8 mm) homozygotes (P=0.04). With the use of multivariate analysis, heterozygosity remained significantly associated with aneurysm size (P=0.03). Conclusions-- The eNOS T-786C SNP distinguishes genetically between small and large ruptured aneurysms. Although not predictive of SAH in the population at large, our data suggest that among persons with known intracranial aneurysms, eNOS T-786C genotype may be a factor influencing the size at which an aneurysm ruptures, a finding that should be taken into consideration along with other anatomic features of the aneurysm.


http://stroke.ahajournals.org/cgi/content/abstract/34/7/1640

Background and Purpose-- A 6-base insertion (6bINS) polymorphism in intron 7 of the endoglin gene (ENG), which codes for a component of the transforming growth factor-β (TGF-β) receptor complex, was reported to be associated with intracranial aneurysm (IA) in a Japanese population. A recent report using a white population could not replicate the association. We tested for this association with high statistical power in our independent Japanese subjects and evaluated the linkage between markers on chromosome 9, which contains ENG and IA. Methods-- The sample for the linkage study comprised 179 individuals with IA in 85 nuclear families, with 104 possible
affected sibpairs. For the association study of the 6bINS polymorphism and 4 single nucleotide polymorphisms (SNPs) in ENG, 172 Japanese patients with IA and 192 control subjects were examined. Results-- There was no evidence of linkage in the vicinity of ENG by analysis of affected sibpairs. The allele frequency of the 6bINS polymorphism was 104 of 344 (30.2%) in the total IA group and 122 of 382 (31.9%) in the control group. The statistical difference in allele frequency between the 2 groups was not significant (x²=0.245, df=1, P=0.620). The power of the present association study was 98.3% at a significance level of 0.05 on the basis of the allele frequencies in the previous study. In addition, no associations between the 4 SNPs in ENG and IA were detected. Conclusions-- We provide evidence that there is no association between the 6bINS polymorphism or 4 SNPs in ENG and IA and that there is no linkage between the ENG locus and IA, indicating that ENG is not a major susceptibility gene for IA in Japanese.


http://stroke.ahajournals.org/cgi/content/abstract/35/1/179

Background and Purpose-- The present study was performed to determine whether antisense inhibition of intercellular adhesion molecule-1 (ICAM-1) protein expression decreases focal ischemic brain damage. Methods-- Male spontaneously hypertensive rats underwent 1-hour middle cerebral artery occlusion (MCAO) and 24-hour reperfusion. Rats were infused with ICAM-1 antisense or control oligodeoxynucleotides (ODNs) (48 nmol/d ICV) or vehicle, starting 24 hours before MCAO and continuing until the time of death. ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) mRNA levels were measured by real-time polymerase chain reaction. ICAM-1 protein knockdown was confirmed by Western blotting. Infarct volume was quantified by the use of cresyl violet-stained brain sections. Neurological deficits were evaluated. Mean arterial blood pressure was recorded by laser Doppler. Tissue penetration of antisense was confirmed by the use of fluorescent ODNs. Results-- Transient MCAO upregulated ICAM-1, but not VCAM-1, mRNA expression in the ipsilateral cortex between 3 and 72 hours of reperfusion. ICAM-1 antisense infusion prevented ischemia-induced ICAM-1 protein expression and reduced total infarct volume (by 53%; P<0.05; 226(+/−)-35 mm3 in control ODN group and 104(+/−)-27 mm3 in antisense ODN group; n=8 each) and mean neurological deficit score (by 44%; P<0.05; 2.4 in control ODN group and 1.3 in antisense ODN group; n=8 each). Neither control nor antisense ODN had any effect on mean arterial blood pressure and the physiological parameters monitored during MCAO. Compared with noninfused control, intracerebroventricular infusion of artificial cerebrospinal fluid or antisense or sense ODN had no significant effect on the regional cerebral blood flow changes that accompanied ischemia and reperfusion. Conclusions-- Increased ICAM-1 expression is implicated in the pathogenesis of focal ischemia since ICAM-1 protein knockdown decreased ischemic brain damage. The mechanism by which ICAM-1 inhibition offers neuroprotection is independent of blood pressure modulation.


http://stroke.ahajournals.org/cgi/content/abstract/34/7/1803

Background and Purpose-- Ischemic injury in neurons can be strongly reduced by a preceding sublethal ischemic episode, of which the mechanism is poorly understood. Although changes in the expression of apoptosis-related proteins (Bcl-2, Bcl-xl, and Bax) have been considered to be crucially important in ischemic injury, the roles these proteins play in ischemic preconditioning induced by sublethal forebrain ischemia have not been elucidated. Therefore, we investigated the
transcription and expression of Bcl-2, Bcl-xl, and Bax in striatum of mice subjected to sublethal forebrain ischemia and lethal ischemia, with or without ischemic preconditioning. Methods--Sublethal forebrain ischemia was induced in C57Black/Crj6 (C57BL/6) mice by 6 minutes of bilateral common carotid artery occlusion. The transcription and expression of Bcl-2 family genes were detected by reverse transcription-polymerase chain reaction, Western blot, and immunofluorescent staining. Results--No detectable neuronal loss was induced in striatum by 6 minutes of bilateral common carotid artery occlusion. Transcription and expression of Bcl-2 and Bcl-xl were increased after sublethal forebrain ischemia, which attenuated the DNA fragmentation induced by lethal ischemia. The transcription and expression of Bax remained unchanged. Conclusions--Upregulation of Bcl-2 and Bcl-xl but not Bax may have a role in protective ischemic preconditioning. This result indicates a potential strategy for further ischemic neuronal injury therapies.


http://stroke.ahajournals.org/cgi/content/abstract/34/4/892

Background and Purpose--We sought to test the linkage of familial intracranial aneurysms (FIAs) to the ELN (elastin) locus in chromosome 7q11 reported previously. Methods--Intracranial aneurysm (IA) probands were searched from patient records or neurosurgeons' recalls in collaborating hospitals. Members of the participating probands' families who had unknown affection status were screened by MR angiography and diagnosed by digital subtraction angiography. Inclusion criteria of families for genetic analyses were as follows: at least 3 alive affected members or 2 alive affected members with at least 1 unaffected member (>60 years). Linkage to the ELN locus was tested with the use of GENEHUNTER by parametric and nonparametric methods. To exclude false-negatives in the linkage analysis, the lowest 5% limits of logarithms of the odds (LOD) and nonparametric LOD (NPL) scores for individual families and for the total set of families were simulated on assumption that the ELN locus is linked to FIAs. Results--Questionnaires were sent to 885 patients, and 563 responded. Seventy-nine probands were positive for family history. One hundred thirty-four family members of unknown affection status were screened. A total of 14 families with 64 members met the criteria. Linkage to the ELN locus was discarded in 11 families and was inconclusive for 3 families. The total LOD and total NPL scores for 14 families were -8.04 and -0.643, respectively. Our conclusion did not change even when the values of penetrance were changed or only affected members were analyzed. Conclusions--The majority of aggregated IA Japanese families may not have a genetic linkage to chromosome 7q11.


http://stroke.ahajournals.org/cgi/content/abstract/35/2/443

Background and Purpose--The collagen (alpha)2(I) gene (COL1A2) on chromosome 7q22.1, a positional and functional candidate for intracranial aneurysm (IA), was extensively screened for susceptibility in Japanese IA patients. Methods--Twenty-one single nucleotide polymorphisms (SNPs) of COL1A2 were genotyped in genomic DNA from 260 IA patients (including 115 familial cases) (mean age, 59.9 years) and 293 controls (mean age, 61.6 years). Differences in allelic and genotypic frequencies between the patients and controls were evaluated with the (chi)2 test. Circular dichroism spectrometry was monitored with collagen-related peptides that mimic triple-helical models of type I collagen with Ala-459 and Pro-459 to estimate the conformation and stability of alterations. Results--Significant genotypic association in the dominant model was
observed between an exonic SNP of COL1A2 and familial IA patients (χ²=11.08; df=1; 
P=0.00087; odds ratio=3.19; 95% CI, 2.22 to 6.50). This SNP induces Ala to Pro substitution at 
amino acid 459, located on a triple-helical domain. Circular dichroism spectra showed that the 
Pro-459 peptide had a higher thermal stability than the Ala-459 peptide. Conclusions-- The 
variant of COL1A2 could be a genetic risk factor for IA patients with family history.

Structure (2)

Characterization Of CBM36: A New Family of Calcium-Dependent Carbohydrate Binding 

http://www.sciencedirect.com/science/article/B6VSR-4CVG586-F/2/5d752b4ba30cb3d79912fe5ad262db62

The enzymatic degradation of polysaccharides harnesses multimodular enzymes whose 
carbohydrate binding modules (CBM) target the catalytic domain onto the recalcitrant substrate. 
Here we report the ab initio structure determination and subsequent refinement, at 0.8 A 
resolution, of the CBM36 domain of the Paenibacillus polymyxa xylanase 43A. Affinity 
electrophoresis, isothermal titration calorimetry, and UV difference spectroscopy demonstrate that 
CBM36 is a novel Ca2+-dependent xylan binding domain. The 3D structure of CBM36 in complex 
with xylotriose and Ca2+, at 1.5 A resolution, displays significant conformational changes 
compared to the native structure and reveals the molecular basis for its unique Ca2+-dependent 
binding of xylooligosaccharides through coordination of the O2 and O3 hydroxyls. CBM36 is one 
of an emerging spectrum of carbohydrate binding modules that increasingly find applications in 
industry and display great potential for mapping the "glyco-architecture" of plant cells.


Background: The Ca2+ binding apoptosis-linked gene-2 (ALG-2) protein acts as a proapoptotic 
factor in a variety of cell lines and is required either downstream or independently of caspases for 
apoptosis to occur. ALG-2 belongs to the penta-EF-hand (PEF) protein family and has two high-
affinity and one low-affinity Ca2+ binding sites. Like other PEF proteins, its N terminus contains a 
Gly/Pro-rich segment. Ca2+ binding is required for the interaction with the target protein, ALG-2 
interacting protein 1 (AIP1). Results: We present the 2.3 A resolution crystal structure of Ca2+- 
loaded des1-20ALG-2 (aa 21-191), which was obtained by limited proteolysis of recombinant 
ALG-2 with elastase. The molecule contains eight [alpha] helices that fold into five EF-hands, 
and, similar to other members of this protein family, the molecule forms dimers. Ca2+ ions bind to 
EF1, EF3, and, surprisingly, to EF5. In the related proteins calpain and grancalcin, the EF5 does 
not bind Ca2+ and is thought to primarily facilitate dimerization. Most importantly, the 
conformation of des1-20ALG-2 is significantly different from that of calpain and grancalcin. This 
difference can be described as a rigid body rotation of EF1-2 relative to EF4-5 and the dimer 
interface, with a hinge within the EF3 loop. An electron density, which is interpreted as a
hydrophobic Gly/Pro-rich decapeptide that is possibly derived from the cleaved N terminus, was found in a hydrophobic cleft between these two halves of the molecule. Conclusions: A different relative orientation of the N- and C-terminal halves of des1-20ALG-2 in the presence of Ca2+ and the peptide as compared to other Ca2+-loaded PEF proteins changes substantially the shape of the molecule, exposing a hydrophobic patch on the surface for peptide binding and a large cleft near the dimer interface. We postulate that the binding of a Gly/Pro-rich peptide in the presence of Ca2+ induces a conformational rearrangement in ALG-2, and that this mechanism is common to other PEF proteins.

Surgery (10)


http://www.sciencedirect.com/science/article/B6WXC-4CHGDDDJS2/c5be7e7b08a17055c75874d14cf2b006

Background There are limited data regarding how many patients with desmoid tumors actually represent cases with underlying familial adenomatous polyposis. Methods A proband presenting with desmoid tumors and several of the family members underwent a detailed family history, genetic (adenomatous polyposis coli [APC] gene sequencing), and upper and lower endoscopic evaluation. Results The proband's initial diagnosis was of a sporadic desmoid tumor. Colonoscopy was entirely normal. However, on subsequent esophagastroduodenoscopy, several gastric polyps were found. The proband's mother subsequently underwent colonoscopy and was found to have multiple colon adenomas. On genetic analysis, a deletion of "T" was identified at codon 2645 of the APC gene in the proband. The proband's mother had a normal APC protein truncation test result. However, on full gene sequencing, the mother was found to harbor the same APC gene mutation. Conclusion A detailed family history and endoscopic and genetic evaluations for patients with desmoid tumors are vital because they may be the sentinel presentation of familial adenomatous polyposis. If confirmed in larger studies, APC full gene sequencing and upper and lower gastrointestinal tract evaluation may need to be part of standard evaluation of patients with abdominal desmoid tumors.


http://www.sciencedirect.com/science/article/B6WXC-4C89FNMK2/2/66bb05751911d5533a5aa1c321853822

Background Trauma causes a release of catecholamines, transforming growth factor-[beta] (TGF-[beta]), and T-helper II cytokines (TH2). Individually, these substances also induce arginase in macrophages. The purpose of this study was to determine the synergistic interactions between isoproterenol, TGF-[beta], and TH2 cytokines on arginase expression in macrophages. Methods Confluent RAW 264.7 macrophages were incubated with various combinations of interleukins 4, 10, and 13 (IL-4, IL-10, IL-13), and TGF-[beta] with isoproterenol over 48 hours. Arginase activity, as well as arginase I expression by Western blot and reverse
transcriptase-polymerase chain reaction, were measured. Results Although isoproterenol, IL-4, IL-10, and IL-13 individually induced arginase, significant synergy between the combination of isoproterenol with either TGF-[beta] or the TH2 cytokines was observed. All cytokines except IL-10 also induced arginase I protein and mRNA. Arginase II protein was detected in cells exposed to IL-10. Conclusions We conclude that isoproterenol synergizes with IL-4, IL-13, and TGF-[beta] to increase arginase I mRNA and protein, as well as arginase activity in RAW 264.7 macrophages. Further, IL-10 synergizes with isoproterenol to increase arginase activity and arginase II protein. These synergistic mechanisms may compete with nitric oxide synthase for arginine substrate, thus shunting away available arginine from nitric oxide production and contributing to cellular immunosuppression observed after trauma.


http://www.sciencedirect.com/science/article/B6WXC-49NPJWT-2/2/248ff07e20e0c93e51077f7a48ff09c8

Background In ischemia/reperfusion (I/R) injury, a massive generation of reactive oxygen species (ROS) after reperfusion is a critical factor. Rac, a member of the Rho GTPase superfamily, plays important roles in the production of ROS and activation of nuclear factor-[kappa]B (NF-[kappa]B) in vitro. However, the exact role of Rac in the ROS production and NF-[kappa]B activation in vivo after I/R is still obscure. Methods We blocked Rac1 activity in the rat liver using adenovirus encoding a dominant negative rac1 mutant (Ad5N17Rac1) and examined whether inactivation of Rac1 could prevent ROS generation in the hepatic I/R injury. Seventy-two hours after the adenoviral infection, hepatic I/R was induced by Pringle's maneuver for 20 minutes, followed by reperfusion in the rats. Results Ad5N17Rac1 infection significantly attenuated ROS production after reperfusion and suppressed the hepatic injury. Furthermore, N17Rac1 suppressed NF-[kappa]B activation and messenger RNA expression of tumor necrosis factor-[alpha] (TNF-[alpha]) and inducible nitric oxide synthetase (iNOS). Ad5LacZ, a control adenovirus, had no effect on the induced hepatic I/R injury, nor did it affect NF-[kappa]B activation. Immunohistochemical analysis of NF-[kappa]B (p65) revealed that translocation of p65 to the nucleus after reperfusion was blocked in many of non-parenchymal cells (NPCs) and in hepatocytes in the Ad5N17Rac1-infected liver. Conclusion We conclude that Rac1 is required in ROS generation and NF-[kappa]B activation after hepatic I/R in vivo, and that inactivation of NF-[kappa]B in NPCs and suppression of ROS generation in NPCs and hepatocytes possibly account for the protective effect of N17Rac1 in this study.


http://www.sciencedirect.com/science/article/B6WXC-4CT11G1-2S/2/3b981e2fc357c056c87b82b316d3a24a

Background. We have previously reported that high extracellular calcium ([Ca2+]o) levels elicited rapid increases in the cytosolic free calcium ([Ca2+]i) and insulin release from human insulinoma cells. In this study we further investigated the mechanism for stimulus-secretion coupling of insulinoma cells exposed to high levels of [Ca2+]o. Methods. Insulinoma tissues were surgically obtained for primary culture. The changes of [Ca2+]i level in response to various agents were monitored by fluorometry. Total RNA was extracted from tissues and subjected to reverse transcription-polymerase chain reaction (RT-PCR) with calcium-sensing receptor (CaR)--specific primers. PCR products were subcloned and sequenced. Results. When [Ca2+]o level was elevated, [Ca2+]i in insulinoma cells was immediately increased. Application of neomycin
abolished the increase in [Ca2+]i level, although extracellular nifedipine and lanthanum chloride
did not affect it. The depletion of intracellular calcium stores with thapsigargin or carbachol
eliminated the increase in [Ca2+]i level. RT-PCR analysis identified the 682 bp product, of which
the sequence was identical to the corresponding regions of human parathyroid CaR.

Conclusions. Intracellular Ca2+ release might be important in insulin release from insulinoma cells after
exposing to high level of [Ca2+]o. CaR could be involved in this mechanism.

Kebebew, E., M. Peng, et al. (2004). "GCMB gene, a master regulator of parathyroid gland development,

http://www.sciencedirect.com/science/article/B6WXC-4F1J2N7-13/2/6281c56585d95ff13cda81d9488ad884

Background The glial cell missing gene, GCMB, encodes a transcription factor, which is a master
regulator of parathyroid development. We postulated that the GCMB gene might play a role in
parathyroid tumorigenesis in hyperparathyroidism. Methods We used real-time quantitative reverse
transcriptase polymerase chain reaction to study GCMB mRNA expression in parathyroid tissue:
normal (n = 3), hyperplastic (n = 16), adenomas (n = 19), and cancers (n = 8). In primary
parathyroid culture, the effect of CaCl2 on parathyroid hormone secretion and GCMB mRNA
expression was studied by using enzyme-linked immunosorbent assay and reverse transcriptase
polymerase chain reaction, respectively. Results GCMB mRNA expression was lower in normal
(0.4 \pm 0.1, mean \pm standard error of mean) parathyroid glands as compared to adenoma (3.5 \pm 1.7), hyperplasia (3.2 \pm 1.3 primary
hyperparathyroidism [n = 11] and 7.6 \pm 4.8 secondary
hyperparathyroidism [n = 5]), and cancer (3.6 \pm 1.3) (P = .001). There was no
difference in the level of GCMB mRNA expression between parathyroid adenoma, hyperplasia,
and cancer. In primary culture of parathyroid adenoma (n = 9) and hyperplasia (n = 2),
parathyroid hormone secretion was increased 2- to 15-fold with low calcium concentration (0.5 to
4.0 mmol/L CaCl2 from 2 to 6 hours, P GCMB mRNA expression was down-regulated with lower
extracellular CaCl2 concentration (P Conclusions GCMB expression is upregulated in abnormal
parathyroid glands of hyperparathyroidism and decreases in response to hypocalcemia. The
GCMB transcription factor might mediate the effect of calcium on parathyroid cell parathyroid
hormone expression/secretion.


http://www.sciencedirect.com/science/article/B6WXC-4CRGF5F-11/2/3ec5c5d2a40ab744ca575ed832303d1d

Background. RET protooncogene mutation analysis is a routinely performed predictive DNA test
in kindreds affected by multiple endocrine neoplasia (MEN) types 2A and 2B and familial
medullary thyroid carcinoma (FMTC), and is a valuable diagnostic tool in newly diagnosed cases
of medullary thyroid carcinoma (MTC). Methods. We tested the suitability of the recently
introduced "cold" single-strand conformational variant (SSCV) technique, which promises rapid,
simple, nonradioactive detection of sequence variants in the identification of germline and
somatic RET mutations. A total of 11 different mutations in exon 10 (codons 609, 611, 618, and
620) and 6 mutations in exon 11 (codon 634) were studied. Results. Conditions were optimized so
that conformational variants were demonstrated for all mutations examined in a single setting for
exons 10 and 11. A novel six base pair (bp) inframe deletion between cysteines 630 and 634 was
detected in a sporadic MTC. This adds to the evidence that not only cysteine deletions and
substitutions but also changes in the spacing between cysteine residues have a pathogenic
Conclusions. Our results indicate that the cold SSCV method offers the advantages of simplicity, time savings, and nonradioactive detection for screening for RET sequence variants in hereditary and sporadic MTCs.


http://www.sciencedirect.com/science/article/B6WXC-4CTDHKF-1M/2/0757dda3c7545865b9ca76e04128f51e

Background. The intensity of discordant xenograft cellular rejection makes it unlikely that safe doses of immunosuppressive drugs will alone be sufficient to permit long-term survival. We have therefore concentrated our efforts on establishing tolerance to xenogeneic organs through lymphohematopoietic chimerism and the elimination of preformed natural antibodies (nAbs).

Methods. Here we report the most recent series of 11 technically successful porcine to nonhuman primate transplantation procedures. In eight experimental animals induction therapy consisted of (1) 3 x 100 cGy nonlethal whole body irradiation (day -6 and day -5) to all animals, (2) horse anti-human thymocyte globulin (day -2, day -1, and day 0) to seven of the animals, (3) 700 cGy thymic irradiation (day -1) to five of the animals, and (4) pig bone marrow infused on day 0 (2-9 x 108/cells/kg). On day 0, just before the renal xenograft, the recipient was splenectomized, and antipig nAbs were removed by means of perfusion of the monkey's blood through either a pig liver (n = 6) or a Gal-[alpha](1,3)-Gal adsorption column (n = 5). Three control animals did not receive this pretransplantation induction therapy but did undergo hemoperfusion and posttransplantation immunosuppression identical to the experimental animals. All 11 recipients were treated after transplantation with cyclosporin A and 15-deoxyspergualin. Recombinant pig-specific growth factors (interleukin-3 and stem cell factor) were given to six experimental animals from day 0 until the termination of the experiment.

Results. Analysis of recipients' sera by means of flow cytometry indicated the effective removal of immunoglobulin M and immunoglobulin G nAbs by either liver perfusion or column adsorption. In the eight experimental animals, nAb titers remained low until death (up to 15 days), but in the three control animals nAb titers increased substantially with time. The longest surviving recipient maintained excellent kidney function with creatinine levels at 0.8 to 1.3 mg/dl throughout its course. Death occurred at day 15 from complications caused by a urinary leak and pancytopenia. Histologic examination of the xenograft revealed only focal tubular necrosis and cytoplasmic vacuolization, with trace amounts of fibrin and C3 in peritubular capillaries. In this animal a fraction of the peripheral blood cells (3%) at day 7 were of pig origin as detected by pig-specific monoclonal antibodies. In addition, colony-forming assays performed on a bone marrow biopsy specimen taken at day 14 indicated that approximately 30% of the relatively few myeloid progenitors detected were of swine origin.

Conclusions. We have demonstrated that our protocol is effective in the prevention of hyperacute rejection and in the maintenance of excellent function of the renal xenograft for up to 15 days. These results also indicate that at least short-term engraftment of the xenogeneic donor bone marrow cells is possible to achieve in this discordant large animal combination. Longer survivals will be required to assess the possible effect of this engraftment on induction of tolerance.


http://www.sciencedirect.com/science/article/B6WXC-4C89FMN-N2/c92919e9ee75cfa1ab6a7d14d8aa1ef4

Background. The anaphylatoxins, C3a and C5a, that are generated during trauma, major surgery,
or infection are potent proinflammatory mediators that increase interleukin (IL-1) cytokine synthesis. We investigated the effects of IL-1 on anaphylatoxin receptor expression in monocytes.

Methods A human monocytic cell line, MONO-MAC-6, was used. C3a and C5a binding sites were assayed by competitive binding. Levels of messenger RNA for the C3a and C5a receptors were analyzed by reverse transcriptase-polymerase chain reaction. Changes of free cytosolic Ca2+ concentration ([Ca2+]i) in response to C3a and C5a were measured.

Results Basal MONO-MAC-6 cell sites for C3a and C5a binding were 10,900 C3aR/cell (Kd = 2.0 nmol/L), 8700 C5aR/cell (Kd = 0.9 nmol/L). IL-1[alpha] increased sites for both C3a (61% increase; P < 0.02+) i increases. IL-1 receptor antagonist blocked the effects of IL-1[alpha] upregulation of anaphylatoxin receptors.

Conclusion These results suggest that there is an additional link between IL-1 and anaphylatoxins to amplify proinflammatory effects through monocytes and macrophages. Although C3a and C5a can increase the monocyte production of IL-1, IL-1 increases monocyte expression of receptors for these anaphylatoxins, which further amplifies inflammation.


http://www.sciencedirect.com/science/article/B6WXC-45SJCX3-B3/2/df66de600af64fadb451ddc83d66bd364

Background. The significance of [alpha]-fetoprotein (AFP) messenger RNA as a surrogate marker for isolated tumor cells in the blood of patients with hepatocellular carcinoma (HCC) is controversial. Our goals were to correlate AFP mRNA with tumor recurrence and overall survival after patients with HCC received curative operations and to analyze AFP mRNA findings in control patients. Methods. In this prospective controlled study, RNA was purified from the blood of 85 patients with HCC before, during, and after therapy and from 116 control patients. Complementary DNA synthesis by reverse transcriptase and polymerase chain reaction amplification was performed with primers specifically for the AFP gene. Patients with HCC were divided into 4 subgroups depending on the therapy performed: (1) orthotopic liver transplantation (OLT), (2) resection, (3) transarterial chemoembolization, and (4) no therapy. Results. AFP mRNA was detected in 28% of the patients with HCC and 3% of the control patients (P = .21 and P = .94, respectively). After the tumor resection, no difference in survival at 2 years was evident in patients who were AFP mRNA positive versus those who were AFP mRNA negative. In the HCC patients who had curative operations (OLT and resection) the sensitivity and specificity of this test for tumor recurrence were 73% and 53%, respectively, excluding surgical mortality. The International Union Against Cancer tumor stages in the subgroups of OLT and resection showed no differences between patients with positive and negative findings (P = .76 and P = .15, respectively). AFP mRNA results and serum AFP levels revealed no correlation (P = .45).

Conclusions. The qualitative measurement of AFP mRNA in the blood of patients with HCC is not a clinically relevant method for determining therapy and prognosis, especially if AFP mRNA is detected during the surgical procedure or any other liver manipulation. (Surgery 2002;131:34-43.)


http://www.sciencedirect.com/science/article/B6WXC-47HXS2-C2/8b0aad1efc47ce7738c4a0ad79ac6d3029

Background. Radioactive iodine is used to identify and treat recurrent and metastatic thyroid cancer of follicular cell origin. Between 30% and 40% of thyroid cancers are either resistant or become resistant to radioactive iodine. Increased sodium-iodide symporter (NIS) and decreased
Pendrin (PDS) activity may be associated with increased radioactive iodine effectiveness. In this investigation the effects of Trichostatin A (TSA), a histone deacetylating inhibitor, on human thyroid NIS and PDS gene expression was investigated. Method. Cell lines from papillary, Hurthle, and follicular cell carcinomas were treated with TSA for 72 hours at concentrations up to 100 ng/mL. NIS and PDS gene expression was determined using quantitative RT-polymerase chain reaction. Results. NIS messenger RNA expression in cell carcinomas was increased 107- (1.8-307) and 217- (5.7-408) fold in papillary, 39- (20-63) and 58- (37-80) fold in Hurthle, and 459- (178-810) and 781- (412-1229) fold in follicular after treatment with 50 and 100 ng/mL of TSA, respectively. PDS messenger RNA expression in cell carcinomas was decreased 0.22- (0.05-0.45) and 0.27- (0.09-0.47) fold in papillary, 0.53- (0.46-0.60) and 0.54- (0.44-0.64) fold in Hurthle, and 0.32- (0.26-0.39) and 0.56- (0.47-0.64) fold in follicular, after the same treatment. Conclusions. In thyroid cancer cell lines, TSA dramatically increased NIS gene expression and reduced PDS expression. The increased NIS expression and reduced PDS expression may make radiiodine therapy more effective in patients with thyroid cancer, especially when the tumors have no or low uptake of radiiodine. (Surgery 2002;132:984-90.)

Systematic and Applied Microbiology (1)


http://www.sciencedirect.com/science/article/B7GVX-4F01192-1/2/3ce6fbe22622d5013bdf3d94eeaa5b6

A slightly creamy, melanogenic, Gram-negative, aerobic bacterium was isolated from seawater sample collected in the Karadag Natural Reserve of the Eastern Crimea, the Black Sea. The novel organism was chemoorganotrophic, had no obligate requirement in NaCl, tolerated to 12% NaCl, grew between 10 and 45 [deg]C, was slightly alkaliphilic, and was not able to degrade starch, gelatin, agar, and Tween 80. 16S rRNA gene sequence-based analyses of the new organism revealed that Oceanimonas doudoroffii ATCC 27123T, Oceanimonas baumanii ATCC 700832T, and Oceanisphaera litoralis DSM 15406T were the closest relatives (similarity around 97%-96%). The G+C content of the DNA of the strain 31-13T was 55.5 mol%. Phosphatidylethanolamine (49.0%), phosphatidylglycerol (41.8%), and diphosphatidylglycerol (9.2%) were the predominant phospholipids. The major fatty acids were 16:0 (24.1%), 16:1[omega]7 (40.3%), and 18:1[omega]7 (29.2%). On the basis of the significant differences demonstrated in the phenotypic and chemotaxonomic characteristics, it is suggested that the bacterium be classified as a novel species; the name Oceanimonas smirnovii sp. nov. is proposed. The type strain is 31-13T (UCM B-11076T=LMG 22147T=ATCC BAA-899T).

The American Journal of Cardiology (4)

The role of chronic viral infection in the etiopathogenesis of idiopathic dilated cardiomyopathy (IDC) has generated considerable research. Enteroviruses were the favorite candidates as etiologic agents of IDC. However, enteroviruses were rarely demonstrated in affected hearts. We investigated whether enteroviral infection persists in the heart and in extracardiac sites, particularly in skeletal muscle, in patients with IDC. Blood and myocardial and skeletal muscle samples were collected at cardiac transplantation from 31 IDC patients, 24 non-IDC heart disease patients, and 3 heart donors. Samples underwent ultrastructural studies and ribonucleic acid (RNA) extraction. RNA was reverse-transcribed, and 2 nested fragments (bps 179 and 126) were amplified in the highly conserved 5' noncoding region of enteroviral genomic RNA. Enteroviral RNA was found in the skeletal muscle of 12 cases, whereas only 4 hearts (2 of which with positive skeletal muscle) were positive. Of the 24 controls, 2 were positive (1 muscle and heart, 1 muscle only). Automated sequencing confirmed the enteroviral nature of the amplified products. Ultrastructural study showed enterovirus-like particles in 4 of the enterovirus-positive muscles, and myopathic changes in all enterovirus-positive cases. Skeletal muscle hosts chronic enteroviral infection in more than one third of patients with sporadic IDC. Two hypotheses may explain this link. Myocardial damage may derive directly from recurrent subclinical heart infections caused by enteroviruses harbored in skeletal muscle. Alternatively, enterovirus-related myopathy may trigger an autoimmune response to antigens shared by muscle and myocardium. Further studies are needed to assess the importance of these, non-mutually exclusive mechanisms in IDC pathogenesis.


To elucidate the etiology of hypertrophic cardiomyopathy (HC) in humans, we analyzed the [delta]-sarcoglycan gene (SG), which is reported to be the causal gene for HC in the Syrian hamster BIO14.6. We performed polymerase chain reaction (PCR) single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses on the [delta]-SG in 102 patients with HC. SSCP was detected in exon 2 of the gene, but not in the other exons. The direct sequencing analysis of exon 2 revealed a C->T substitution at nucleotide residue 84 (TAC->TAT) with no amino acid alteration (Tyr->Tyr). There were no significant differences in allele frequencies of C/T between the patients with HC and the control group. Patients with HC were classified into 4 subgroups: obstructive HC, nonobstructive HC, apical HC, and familial HC. The allele frequency of C/T polymorphism in each of these groups was compared with that of the control group. The obstructive HC group showed a significantly greater frequency of the allele T than in the control group (31.6% vs 15.1%, RR = 2.6, p = 0.023). No other significant differences were observed. Thus, amino acid alteration in [delta]-SG may not be a common cause of HC in Japanese patients.

A polymerase chain reaction (PCR) amplification assay was developed to detect Coxsackievirus B3 ribonucleic acid (RNA) in blood and myocardial tissue of explanted hearts from 40 patients who underwent cardiac transplantation and in 1 normal heart. Twenty-one patients were affected by idiopathic dilated cardiomyopathy of different duration and 19 by coronary artery disease. Coxsackievirus B3 in vitro infected Vero cells and cells infected by related human enteroviruses (Coxsackievirus B2, B4, and poliovirus 1) were used as reaction controls. PCR was performed using 4 pairs of primers homologous to Coxsackievirus B3 sequences. Three sets were located in regions of the genome conserved at nucleotide level between several enterovirus species (replicase gene, 5’ noncoding region), while one was located in a Coxsackievirus B3-specific region (VP1 gene). Total RNA was prepared by acid guanidinium isothiocyanate extraction from tissue stored frozen at -80 [deg]C. One microgram of total RNA was retrotranscribed with either antisense primer or with random hexanucleotide primers and then subjected to 40 cycles of amplification. PCR products were separated by electrophoresis on a 10% polyacrilamide gel, electrotransferred to a nylon membrane and then hybridized to oligonucleotide probes specific for the coxsackievirus B3 genome radiolabeled with radioactive isotope of phosphorous. All pairs of primers yielded specific amplification products when tested on Coxsackievirus B3-infected Vero cells, with a sensitivity of 1 infected cell out of 105 to 106 cells starting from 1 [mu]g total RNA. Primer sets for regions of Coxsackievirus B3 genome highly conserved between related enteroviral species gave positive amplification also when challenged with RNA from cells infected by Coxsackievirus B2, B4 and poliovirus 1. The VP1 gene primer set produced positive amplification only with RNA of Coxsackievirus B3-infected cells. Coxsackievirus B3-specific amplification products were distinguished from those of related enteroviruses by hybridization with specific oligoprobes. However, Coxsackievirus B2, B4 and poliovirus 1-specific PCR products showed positive hybridization if probed with Coxsackievirus B3 genomic probes. All total RNAs from blood and myocardial samples examined by our PCR assay failed to reveal any amplification product that could be related to Coxsackievirus B3 or to enteroviruses in general, after gel electrophoresis and low stringency Southern blot hybridization with the Coxsackievirus B3 specific oligoprobes or the Coxsackievirus B3 genomic probes. The negative results obtained in our series question the hypothesized widespread persistence of enteroviral RNA in hearts with idiopathic dilated cardiomyopathy.

were somewhat more numerous in the myocardium of symptomatic than asymptomatic patients. Our studies are the first to directly detect the HIV genome in purified cardiac myocytes from patients with and without cardiac dysfunction. Our findings do not support a direct role of the virus in myocardial dysfunction. However, the results do suggest that the interstitial dendritic cells may be involved in some manner in the development of cardiac dysfunction observed in HIV-infected patients.


http://www.sciencedirect.com/science/article/B6VHV-3MPJB-Y/2/3c50923f04a78ce54aa1b9b710b0340

Objective: Familial adenomatous polyposis coli (FAP) is an autosomal dominant disease characterized by an early onset of numerous adenomatous polyps of the colon and a high risk of colon carcinoma. The role of the p53 gene in the multistage process of FAP is as yet poorly defined. In the present study, a large family with evidence of polyposis and colon cancer was screened for the mutations of the p53 gene and protein overexpression. Methods: We examined p53 protein expression from individuals with immunohistochemical techniques using monoclonal antibody PAb1801. Polymerase chain reaction products of exons 4-9 of the p53 were examined from individuals by single strand, conformational polymorphism analysis. Results: We could find no evidence of overexpression and mutations of the p53 in any lesion including adenomas and carcinomas. Conclusion: We found that p53 gene alterations do not contribute to the genesis of adenoma or carcinoma of FAP patients for this large family examined.


http://www.sciencedirect.com/science/article/B6VHV-3YMW3JM-17/2/0cd317c89b86788a396026b4c783dea0

OBJECTIVE: Recently a novel DNA virus (TT virus) has been identified in Japan and shown to be associated with elevated aminotransferase levels after blood transfusion. The exact role of TTV in the pathogenesis of liver disease is yet to be established. Our aim was to determine the prevalence and role of TTV in the pathogenesis of elevated transaminases in healthy blood donors in the absence of markers for viral hepatitis A-C. METHODS: Stored sera were collected from 99 healthy blood donors with elevated alanine amino transferase (ALT) values that were discovered at the time of blood donation. A total of 146 samples were obtained from healthy donors with normal ALT values who were used as controls. None of the patients or controls had a history of blood transfusion or had clinical signs of acute or chronic hepatitis. Serological markers for hepatitis A, hepatitis B, and hepatitis C viruses were negative. TTV DNA was amplified and detected using polymerase chain reaction followed by gel electrophoresis. RESULTS: Five of 99 (5%) samples obtained from donors with elevated ALT had TTV DNA detected by PCR, as
compared to one of 146 (0.7%) of those with normal ALT (p = 0.006). Among those with elevated ALT, mean ALT values in patients with TTV (296 +/- 305 U/L) were higher than in patients without TTV (95 +/- 37 U/L), but the difference was not statistically significant (p = 0.08). The two samples with highest ALT values (both >450 U/L) were among the five samples with detectable TTV DNA in serum.

CONCLUSIONS: Although TTV is not likely to explain the majority of elevated ALT cases in otherwise healthy blood donors, TTV infection may potentially be associated with some cases. Based on these findings, we propose that the role of TTV in the pathogenesis of acute and chronic liver diseases merits further investigation.


http://www.sciencedirect.com/science/article/B6VHV-3WDKFDS-1G/2/10bb3579a45fa4077559289927f43cbf

OBJECTIVE: Hepatitis C virus (HCV) is known to be heterogeneous and to circulate as a group of closely related quasispecies in individual patients, although hepatic viral genetic characteristics have not been well documented. METHODS: Matched serum and liver samples were tested by reverse transcription polymerase chain reaction amplification and single stranded conformation polymorphism analysis of the hypervariable portion of the E2/NS1 region of the HCV genome. The number of quasispecies was compared with the amount of HCV RNA, HCV genotyping, and infection with the hepatitis G virus. RESULTS: Sixteen of 40 patients had HCV RNA detectable in serum and liver. The HCV genotype was identical in serum and liver of all but one case. HCV RNA levels were approximately 10-fold higher in liver than serum. The number of HCV quasispecies in serum ranged between two and six (median 3.0) and in the liver between 2 and 19 (median 3.5, mean liver/serum ratio 1 to 6.3, median 1.8). The number of quasispecies in liver was equal to or greater than that in serum in all cases. HGV infection was found in 14 cases and did not influence serum or hepatic levels of HCV RNA. CONCLUSIONS: The number of hepatic HCV quasispecies usually exceeds that in serum, independent of the amount of HCV RNA and HCV genotype. This finding is compatible with clearance of some quasispecies from serum, but not liver, by putative neutralizing antibodies.


http://www.sciencedirect.com/science/article/B6VHV-3Y2N7PH-X/2/becebeb2d28bcd5a6d168977aba9dd8d7f

OBJECTIVE: Numerous investigators have proposed a role for bacteria in biliary lithogenesis. We hypothesized that bacterial DNA is present in gallstones, and that categorical differences exist between gallstone type and the frequency of bacterial sequences. METHODS: Polymerase chain reaction (PCR) was used to amplify bacterial 16S rRNA and uidA (encoding Escherichia coli [E. coli] [beta]-glucuronidase) genes in different types of gallstones. PCR products were sequenced. RESULTS: Bacterial 16S rRNA and uidA DNA sequences in E. coli were detected in all brown pigment, common bile duct, and mixed cholesterol gallstones (n = 14). In contrast, only one (14%) of seven pure cholesterol gallstones yielded a PCR product. Most (88%) mixed cholesterol gallstones yielded PCR amplification products from their central, as well as their outer, portions. Sequenced products possessed 88-98% identity to 16S rRNA genes of E. coli and Pseudomonas species. CONCLUSIONS: Bacterial DNA sequences are usually present in mixed cholesterol (to 95% cholesterol content), brown pigment, and common bile duct, but rarely in pure cholesterol gallstones. The presence of bacterial [beta]-glucuronidase is also suggested. The role of bacteria and their products in the formation of mixed cholesterol gallstones, which comprise the
majority of cholesterol gallstones, warrants further study.


http://www.sciencedirect.com/science/article/B6VHV-3WM59KY-N/2/d9739ce7cbd5a942f47613b70816f241

OBJECTIVE: Whether Helicobacter pylori infection and use of nonsteroidal antiinflammatory drugs (NSAIDs) are independent risk factors for ulcerogenesis remains unclear. We undertook this study to evaluate H. pylori isolates from gastric ulcer patients to determine whether the genotype of the infecting isolate could be correlated with the use or nonuse of NSAIDs. METHODS: Fifty-two patients presenting with gastric ulcer and infected with H. pylori were included; 26 patients were taking NSAIDs or aspirin (ASA) regularly at the time of ulcer diagnosis. Polymerase chain reaction (PCR) was employed to assess the presence and mosaicism of the following H. pylori genes: cagA, vacA, iceA, and picB. RESULTS: We found no statistical differences in the presence of these genes in H. pylori isolates from gastric ulcer patients taking or not taking prescription NSAIDs or ASA. A 297-bp fragment of the cagA gene was detected in 96% of the isolates from the NSAID and ASA users and 100% from the non-NSAID users (p = 1.0). A larger and more variable region of the cagA gene was detected more frequently among the isolates from non-NSAID users than those from NSAID users (p = 0.05). Ninety-two percent of the isolates were identified as vacA genotype s1. The dominant vacA subtype was s1b, 76.9% and 65.4% in isolates from non-NSAID-taking or NSAID-taking patients, respectively (p = 0.4). iceA1 genotype was not correlated with gastric ulcer as this allele was only detected in 17.3% of all isolates. CONCLUSIONS: No significant differences in the presence of the candidate virulence genes vacA, cagA, picB, or iceA were detected in isolates from gastric ulcer patients taking prescription NSAIDs or ASA, compared with those not taking these drugs, indicating that single gene presence does not allow discrimination of isolates that may be important in NSAID-induced ulcerogenesis. A variable region of the cagA gene was more frequently detected in isolates from patients not taking NSAIDs or ASA, suggesting that this gene may be modified by NSAID- or ASA-related factors or that certain strains may be selected for in patients taking these medications.


http://www.sciencedirect.com/science/article/B6VHV-3VS1R98-M/2/b1b44137982511aac4a0f83d1c3f0b38

Objective: Few studies have examined the genetic relationships of Helicobacter pylori strains affecting family members. Our aim was to do so. Methods: We characterized H. pylori isolates obtained from members of a single family presenting with various gastroduodenal diseases to examine H. pylori bacterial genetic similarity. Endoscopic evaluation with gastric mapping was performed on each individual to establish clinical and histological disease. Genomic DNA extracted from each H. pylori isolate was used to generate DNA fingerprints for each strain by REP-PCR. vacA genotypes and cagA presence were established by PCR. Results: Gastrointestinal diseases among the five members of this family included gastric adenocarcinoma in a 52-yr-old man (index patient), gastric MALT-lymphoma in the 73-yr-old mother; intestinal metaplasia (IV) and atrophic gastritis in the 48-yr-old brother; intestinal metaplasia (I-III) in the 47-yr-old brother, and a duodenal ulcer scar in the 42-yr-old sister. REP-PCR DNA fingerprints of H. pylori isolates from the index patient, his mother, and both of his brothers were identical or highly similar. By contrast, the H. pylori DNA fingerprint from the sister was markedly different from the
H. pylori DNA fingerprints from the other family members. All isolates had the genotype cagA-positive and vacA s1b/m1 mosaic genotype. Conclusions: The DNA fingerprints of H. pylori strains obtained from members of this family with malignancy or premalignant histological disease were identical or highly similar and markedly different from the H. pylori DNA fingerprint from the sibling with duodenal ulcer disease. All H. pylori isolates within the family possessed genetic markers of enhanced virulence (presence of the cagA gene and vacA s1/m1 mosaicism). In addition to host genetics and environmental factors, these findings suggest that infection with genetically similar H. pylori strains is a significant factor in determining the clinical outcome of an infection with H. pylori.


http://www.sciencedirect.com/science/article/B6VHV-42SPMRX-K/2/2e838906af31fee3f1f94d28d783a5e8

OBJECTIVE: Both bacterial virulence factors and the pattern of Helicobacter pylori (H. pylori) gastritis may contribute to the development of clinically relevant gastroduodenal disease. The aim of our study was to investigate the frequency of H. pylori vacA alleles, iceA, and cagA, and the pattern of gastritis in patients with gastric cancer (GC), gastric lymphoma (MALT), duodenal ulcer (DU), and functional dyspepsia (FD). METHODS: H. pylori was cultured from 141 patients (34 GC, 26 MALT, 49 DU, 32 FD). Allelic variants of vacA and iceA, and cagA were identified by polymerase chain reaction. Antrum and corpus biopsies were obtained for assessment of gastritis according to the updated Sydney System. RESULTS: The vacA s1,m1 genotype was more frequently detected in H. pylori from GC patients (70.6%) than from MALT, DU, and FD patients (p iceA1 and cagA did not differ among the groups. The proportion of patients with severe gastritis in the corpus was significantly higher in patients with GC and MALT compared with patients with DU (p CONCLUSIONS: In a German patient population, only the vacA s1,m1 genotype of H. pylori is associated with GC, and therefore may be useful to identify infected patients being at an increased risk for GC.


http://www.sciencedirect.com/science/article/B6VHV-436FB4H-1F/2/fcbdc38fc55708e7c093603423c751c

OBJECTIVES: Telomerase is highly activated in a variety of malignant neoplasms including colon cancer. Among the major components of telomerase, human telomerase reverse transcriptase (hTERT) is thought to regulate telomerase activity. To assess the importance of telomerase for the diagnosis of colorectal cancer, we measured the expression of hTERT mRNA and telomerase activity in a large series of 140 colorectal cancers, 140 adjacent normal tissues, and 20 adenomas. METHODS: The expression level of hTERT was measured quantitatively by competitive reverse transcriptase-polymerase chain reaction (RT-PCR), and telomerase activity was examined by telomeric repeat amplification protocol (TRAP) assay in the same samples. RESULTS: The median expression level of hTERT mRNA in carcinomas was significantly higher than that in either adenomas or normal tissues. The median level of hTERT in adenomas was significantly higher than that in normal tissues. Telomerase activities in carcinomas were significantly higher than those in either adenomas or normal tissues. Telomerase activities in adenomas were also significantly higher than those in normal tissues. Furthermore, the relative expression levels of hTERT mRNA in adenomas and carcinomas were
significantly correlated with the relative telomerase activities; the Spearman rank correlation was 0.53 ($p = 0.021$) and 0.18 ($p = 0.031$), respectively.

**CONCLUSIONS:** Our data suggest that determination of hTERT mRNA by competitive RT-PCR is superior in quantitative accuracy and sensitivity and would support the importance of telomerase activity for the diagnosis of colorectal cancer.


OBJECTIVE: Hepatitis C virus (HCV) is the major causal agent of non-A, non-B hepatitis and the leading indication for liver transplantation worldwide. The emerging field of immunogenetics has confirmed the significant role of heritability in host immune responses to infectious pathogens. Both the major and non-major histocompatibility complex genes are increasingly identified as candidate genes hypothesized to influence the susceptibility to, or the course of, a particular disease. We hypothesized that polymorphisms within the major histocompatibility complex class III region that encode for tumor necrosis factors (TNF)-[alpha] and TNF-[beta] might be predictive of response to antiviral therapy in patients with chronic hepatitis C.

**METHODS:** A total of 155 subjects, including 110 HCV-seropositive individuals undergoing antiviral therapy and 45 ethnically similar HCV-negative controls, were studied. The HCV-positive patients had undergone antiviral treatment with either interferon monotherapy ($n = 73$) or in combination with ribavirin ($n = 37$) and were categorized as either nonresponders, sustained responders, or relapsers. Sixty (55%) patients had genotype 1 (1a or 1b). Genomic DNA was extracted, followed by polymerase chain reaction amplification and sequencing for two promoter TNF-[alpha] variants (at positions -238 and -308), as well as restriction fragment length analysis for four polymorphic loci within the TNF-[beta] gene (NcoI, TNFc, aa13, aa26).

**RESULTS:** Although there was a trend toward higher frequency of the A allele in the TNF 238 promoter among HCV-infected patients (12% vs 4%), there were no significant differences in the distribution of the genotypic polymorphisms between patients and controls. Patients with the TNF 238 A allele had higher pretreatment viral loads as compared with patients homozygous for the wild type allele ($7.2 \times 10^6 \pm 4.2 \times 10^6$ copies/ml vs $3.8 \times 10^6 \pm 0.34 \times 10^6$ copies/ml, $p = 0.03$). However, there was no association between TNF genetic markers, including multiple haplotypic combinations, and response to therapy. In addition, there was no correlation with these polymorphic loci and histological severity of liver disease.

**CONCLUSIONS:** Although previous work has suggested potential roles for TNF in the pathogenesis of HCV infection, we were unable to identify any link between TNF genetic polymorphisms and histological severity or response to antiviral therapy.


Objective The aim of this pharmacogenomics study was to investigate the influence of different cytochrome P450 (CYP) genotypes in Helicobacter pylori eradication therapy. Methods The study involved 143 consecutive Italian Caucasian patients with H. pylori infection diagnosed and treated with 1-wk triple therapy according to European Helicobacter Pylori Study Group guidelines. Using human genomic DNA, CYP2C19 (*2 and *3) and CYP3A4 alleles (*1B, *2, and *3) were evaluated by polymerase chain reaction-restriction fragment length polymorphism assays and
confirmed by sequencing the amplicons. Results According to the endoscopy-based gold standard, 93 patients achieved H. pylori eradication. Regarding CYP2C19 genotype, the 50 patients who remained infected were all homozygous or heterozygous extensive metabolizers (homEM or hetEM). Carriers of homEM fared significantly less well than those of hetEM; homEM genotype was also predictive of failure at univariate/multivariate analysis. Carriers of CYP3A4 polymorphisms achieved favorable eradication rates similar to patients bearing CYP2C19. All four patients with single CYP3A4*2 polymorphism achieved eradication, and only 29% (5/17) of all CYP3A4*1B carriers did not achieve eradication. All nine patients carrying CYP3A4 polymorphisms in the CYP2C19 hetEM subgroup were cured, suggesting the possibility of a positive synergism between CYP3A4 and CYP2C19. Conclusions This first pharmacogenomics study on the influence of different CYP genotypes on H. pylori therapy suggests that, as in Asian populations, CYP2C19 genotype patterns are probably also relevant in Caucasians receiving H. pylori eradication regimens that include omeprazole. The possibility of a favorable drug interaction mediated by CYP2C19 and CYP3A4 requires investigation.


http://www.sciencedirect.com/science/article/B6VHV-4B1S6DM-12/2/c3c80e0c9815b9fe65c1ce814c8f34fe

Objectives NOD2/CARD15 variants have recently been shown to be associated with Crohn's disease (CD). No analysis of NOD2/CARD15 gene variants has so far been reported in pediatric patients. Therefore, our aim was to analyze NOD2/CARD15 gene variants in children with CD and to perform genotype-phenotype analyses. Methods We studied 101 children with CD and 136 healthy controls. Detailed phenotypic information was obtained from each patient. Patients were genotyped for the three NOD2/CARD15 variants R702W (single nucleotide polymorphism 8 [SNP8]), G908R (SNP12), and L1007fs (SNP13), and genotype-phenotype correlations were performed. Results We found 33 NOD2/CARD15 mutations in 29 of 101 patients (29%). The frequency of NOD2 variation was 31% in white (n = 87) compared with 11% in controls (\(\chi^2\) = 14; \(p = 0.0001; OR = 3.7; 95\% CI = 1.7-7.8\)). Four white patients but not control subjects were compound heterozygotes. NOD2/CARD15 variants were significantly associated with ileal disease (\(\chi^2 = 4.5; p = 0.03; OR = 5; 95\% CI = 0.9-35.9\)). Of the children with NOD2/CARD15 variants, 44% were 2\(=8.7; p = 0.003; OR = 4.5; 95\% CI = 1.4-14.4\)). Similar trends were observed for height but they did not reach statistical significance. Conclusions Our results demonstrate that: 1) the three NOD2/CARD15 variants confer risk to CD in children; 2) NOD2/CARD15 variants are associated with ileal disease in children as in adults; and 3) NOD2/CARD15 variants are associated with lower weight percentiles at diagnosis in children and a tendency toward lower height percentile, suggesting an association between growth in children with CD.


http://www.sciencedirect.com/science/article/B6VHV-45R57F5-K/2/0f407e48dc8f24dc69e4af18d8e0177b

OBJECTIVE: The pathogenesis of chronic pancreatitis (CP) is poorly understood. Genetic studies revealed mutations in the cationic trypsinogen gene and an increased frequency of cystic fibrosis gene mutations in patients with CP. Recently, a point mutation (N34S) in the gene encoding the
serine protease inhibitor, Kazal type 1 (SPINK1), was found in approximately 20% of patients with CP. The aim of our study was to determine the frequency of the N34S SPINK1 gene mutation in a well-defined patient cohort with idiopathic CP (ICP) and to compare the incidence with healthy controls. In addition, we investigated the impact of this mutation on the long-term course of CP.

METHODS: Fourteen patients with early-onset and four patients with late-onset CP of our well-defined pancreatitis cohort were enrolled in the present study, and 397 healthy individuals served as a control population. Coding exonic and the flanking intronic sequences of SPINK1 were investigated by direct DNA sequencing. The mutations found were confirmed by melting curve analysis. In addition, the N34S mutation was detected by analyzing the DNA fragments generated by digestion with restriction enzyme TspR I. Clinical data of patients with the N34S mutation were compared with those without mutations.

RESULTS: The N34S mutation was detected in six of 14 (43%) patients with early-onset ICP. One patient was homozygous, and five patients were heterozygous for this mutation. The N34S mutation in a heterozygous state was found in four of 397 healthy controls (1.0%). The different allele frequency observed (seven of 28 vs four of 794) was significant (odds RATIO = 66, 95% CI = 18-242, p

CONCLUSIONS: Our results indicate that the N34S mutation in the SPINK1 gene is strongly associated with ICP, especially with the early-onset type. The natural course is similar in patients with mutations compared with SPINK1 mutation-negative patients. The N34S mutation may easily be screened for by restriction digestion with TspR I.


http://www.sciencedirect.com/science/article/B6VHV-3Y2N7PH-17/2/bac4101909ec6c734dce78590c06014b

OBJECTIVE: Recently, TT virus (TTV), associated with posttransfusion hepatitis, was discovered. Prevalence of TTV infection in maintenance hemodialysis (HD) units and its pathogenicity to liver was investigated.

METHODS: A total of 115 patients on HD were assessed for presence of serum TTV. DNA was purified from sera, and nested polymerase chain reaction was done for the detection of TTV DNA.

RESULTS: TTV was detected in 59 patients on HD (51.3%), as compared with healthy blood donors (15 of 91 [16.5%], p = 15 IU/L in TTV-positive patients (14 of 18) than in TTV-negative patients (five of 15) (p

CONCLUSIONS: TTV infection is remarkably prevalent in patients on HD and in healthy blood donors. It is suggested that TTV generally does not cause liver disease by itself, but there remains the possibility that TTV may aggravate liver disease caused by HCV.


http://www.sciencedirect.com/science/article/B6VHV-40PXN7S-10/2/a7327a3d7e7e213b378c914ca17356cf

OBJECTIVE: TT virus (TTV) has been identified as a candidate agent of non-A-E hepatitis virus. We investigated superinfection of TTV in patients with chronic hepatitis C and studied the susceptibility to interferon (IFN) treatment and its association with liver disease caused by hepatitis C virus (HCV).

METHODS: TTV DNA was examined using the seminested polymerase chain reaction (PCR), and its virus level was measured by the real-time fluorometric PCR.

RESULTS: TTV DNA was detected in 20 of 102 (19.6%) patients examined. There was no significant difference in the alanine aminotransferase (ALT) level between patients with or without TTV DNA. Quantitative analysis of HCV RNA and TTV DNA revealed no correlation between virus levels in HCV/TTV-coinfected patients. Both TTV and HCV were sensitive to IFN therapy.
Complete response to IFN with a sustained loss of viremia for 24 wk after completion of IFN treatment was found in 11 of 20 (55%) patients with respect to TTV DNA and in five of 20 (25%) patients with respect to HCV RNA. The mean pretreatment HCV RNA level was significantly lower in the complete-response cases than in the no-response cases, but there was no significant difference in the pretreatment TTV DNA levels between them. ALT normalization resulting from IFN therapy was not attributable to the eradication of TTV DNA but was attributable to that of HCV RNA. Superinfection by TTV did not influence the effect of IFN against HCV. No specific TTV genotype correlating with IFN sensitivity was found. CONCLUSIONS: These results suggest that TTV infection stands independent of HCV infection, with no influence on liver injury as a result of HCV infection.


http://www.sciencedirect.com/science/article/B6VHV-40YYFTJ-J/2/068a20f648c4209f450000d87dc2940f

OBJECTIVE: Frequent P53 mutations and Ki-ras codon 12 point mutations have been reported in pancreatic cancer. Pancreatic cancer often recurs in the liver and/or lymph nodes shortly after a surgical resection. The purpose of this study is to elucidate the occurrence of microcirculating cancer cells and micrometastasis in pancreatic cancer. METHODS: P53 mutations and Ki-ras codon 12 point mutations were examined in the main tumor, liver, portal vein, and peripheral arterial blood, and para-aortic lymph nodes of patients with pancreatic cancer using molecular examinations. RESULTS: P53 mutations in the main tumor were present in nine (29%) of 31 patients with pancreatic cancer, whereas a Ki-ras codon 12 point mutation was evident in 18 (62%) of 29 examined patients. The peripheral arterial and portal vein blood and liver were positive for gene abnormalities in one (5%) of 21, in none (0%) of 19, and in one (1%) of 20, respectively. A P53 mutation in the main tumor was evident in none (0%) of seven stage I or II carcinomas and in nine (38%) of 24 stage III or IV cases, whereas a Ki-ras codon 12 point mutation was present in four (67%) of six stage I or II cases and in 14 (61%) of 23 stage III or IV cases. In addition, 15 (71%) of 21 patients with gene abnormalities (Ki-ras codon 12 point and/or p53 mutation) in the main tumor showed lymph node metastasis at surgery, whereas five (42%) of 12 without gene abnormalities did not demonstrate lymph node metastasis. Two (29%) of six patients with gene abnormalities in the main tumor and without metastatic disease at surgery developed liver metastasis within 6 months after surgery, whereas all five (100%) without the gene abnormalities and metastatic disease at surgery did not develop the metastasis, with the sensitivity being 100%, specificity 44%, the predictive value of the positive test 36%, and the predictive value of the negative test 100%. Two patients who had gene abnormalities in the para-aortic lymph node were free from histopathological metastasis and these two patients developed para-aortic lymph node metastasis within 6 months after surgery. CONCLUSIONS: A molecular examination of Ki-ras codon 12 and p53 mutations therefore enables us to predict, to some degree, the occurrence of liver and lymph node metastasis in pancreatic carcinoma.


http://www.sciencedirect.com/science/article/B6VHV-3XJTJDC-1P/2/b3307d268d21cfc9c7cf3b60e2059

OBJECTIVE: The TT virus (TTV) is a novel DNA virus that has recently been identified. The clinical significance of TTV infection in patients with chronic hepatitis C has not been determined.
The aim of this study was to determine the prevalence and possible role of TTV in a well characterized population with chronic hepatitis C infection. METHODS: Ninety patients with chronic HCV and known time of HCV acquisition were selected from approximately 250 patients followed at our institution. Characteristics including age, sex, histology, and length of disease were recorded. Direct sequencing of the NS5 region was used for HCV genotyping. TTV DNA detection was based on PCR. RESULTS: TTV infection was present in 24 of 90 (27%) HCV patients. Patients were divided into four groups based on stage of disease: chronic hepatitis (CH, 29 patients), compensated cirrhosis (CC, 17 patients), decompensated cirrhosis (DC, 28 patients), and hepatocellular carcinoma (HCC, 16 patients). TTV was present in 2/29 (7%), 2/17 (12%), 11/28 (39%), and 9/16 (56%) in those with CH, CC, DC, and HCC respectively. TTV was significantly more prevalent among those with advanced disease (DC and HCC) compared to those with stable disease (CH and CC; p = 0.001). Mean age, sex, and the time from exposure to HCV to development of complications were similar in TTV-positive and -negative patients. TTV infection was more common in patients infected with HCV genotype 1b. Univariate analysis showed that length of HCV infection, HCV genotype 1b, and TTV infection were important in predicting the stage of liver disease in HCV patients. However, after adjusting for length of HCV infection, TTV but not HCV genotype was important in predicting the stage of liver disease. CONCLUSIONS: We conclude that 1) TTV infection is common in patients with chronic HCV; 2) TTV infection is more prevalent among patients with advanced HCV-associated liver disease (DC and HCC) than in those with stable disease (CH and CC); and 3) TTV infection is more common in patients with HCV genotype 1b but is independent from genotype in predicting the stage of HCV-associated liver disease.


http://www.sciencedirect.com/science/article/B6VHV-47C72J8-10/2/9bbbc5282aaa0764a97e0d0fa87f552

OBJECTIVE: Recent reports indicate that allelic variants in NOD2/CARD15 are associated with Crohn's disease (CD) susceptibility, and that homozygosity or compound heterozygosity at this locus for any of three recently defined sequence variants confers a greatly increased risk of CD. These sequence changes include two missense mutations, R702W and G908R, and a frameshift insertion, 1007insC. The aim of this study was to determine the frequency of these NOD2/CARD15 variants in familial and sporadic CD patients in the Ashkenazi population and to determine their effects on disease susceptibility and age of disease onset (AOC). METHODS: Allele and genotype frequencies of these three variants were determined in 481 CD patients of Jewish descent and 110 Jewish controls; 169 patients had a family history of CD, and 312 were "sporadic" cases. Variants were detected by polymerase chain reaction using allele-specific primers labeled with fluorescent dye. RESULTS: Familial cases had a significantly higher frequency of the G908R variant than sporadic cases (0.127 vs 0.059, p = 0.0003) and correspondingly, a significantly higher proportion of homozygotes and compound heterozygotes (11.8% vs 4.5%, p = 0.0027). Homozygotes and compound heterozygotes had an OR for CD of 14.6 for familial cases and 5.1 for sporadic cases. There was no increased risk of CD for simple heterozygotes. The AOC was significantly lower for CD patients who were homozygotes and compound heterozygotes for NOD2/CARD15 (17.5 vs 22.4 yr, p = 0.04), but only for familial cases. CONCLUSIONS: NOD2/CARD15 contributes more to CD susceptibility in familial cases than in sporadic cases, and to an earlier AOC. There is no increased risk of CD for individuals carrying only a single copy of these NOD2/CARD15 variants, whereas individuals carrying two copies have a 5-15-fold increased risk. The penetrance of the NOD2/CARD15 mutations was estimated at less than 1%.

http://www.sciencedirect.com/science/article/B6TDC-40SFT42-1N/2/2ecb6c8539a3cd36e11d6baa3b07fe10


Background. The presence of tumor cells in the blood stream is considered evidence of a high risk of distant organ metastasis. We examined the usefulness of telomerase activity in peripheral blood polymorphonuclear cells as an indicator of distant metastasis in patients with esophageal squamous cell carcinoma.

Methods. Telomerase activity was measured in the peripheral blood mononuclear cell and polymorphonuclear cell fractions obtained from blood samples of healthy volunteers mixed with squamous cell carcinoma cell lines, and cell distribution was analyzed by flow cytometry. Then telomerase activity of forty-two polymorphonuclear cell fractions obtained from esophageal squamous cell carcinoma patients was measured.

Results. Telomerase activity was detected in polymorphonuclear cell fractions and cell distribution analysis revealed the presence of esophageal squamous cell carcinoma cells. Organ metastasis was detected in 7 (78%) of the 9 patients with telomerase-positive polymorphonuclear cell fractions as opposed to only five (15%) of the 33 with telomerase-negative cases, and there was a significant positive correlation between telomerase activity and organ metastasis (p Conclusions. Measurement of telomerase activity in the polymorphonuclear cell fractions is useful for identifying a high risk group for distant organ metastasis in patients with esophageal squamous cell carcinoma.


http://www.sciencedirect.com/science/article/B6T11-3YC05G7-5/2/a0d5fc1c1472117f1b3426c4efafa3a0

Background. Adenovirus (Ad) vector-mediated gene therapy strategies have emerged as promising modalities for the "biological revascularization" of tissues. We hypothesized that direct intramyocardial, as opposed to intracoronary, administration of an Ad vector coding for the
vascular endothelial growth factor 121 cDNA (AdGVVEGF121.10) would provide highly focal Ad genome levels, and increases in VEGF, ideal for inducing localized therapeutic angiogenesis.

Methods. Persistence and regional distribution of the vector were assessed by TaqMan real-time quantitative polymerase chain reaction technology and enzyme-linked immunosorbent assay, after intramyocardial AdGVVEGF121.10 in the rat, and either intramyocardial or intracoronary (circumflex territory) vector in Yorkshire swine. Based on these results, we assessed the focal nature of the improved cardiac blood flow in a previously reported porcine myocardial ischemia model.

Results. Intramyocardial delivery of AdGVVEGF121.10 in the rat resulted in local persistence of the Ad genome that decreased 1,000-fold over 3 weeks, with peak myocardial VEGF expression 24 to 72 h after vector delivery. After intramyocardial AdGVVEGF121.10 in the circumflex distribution of pigs, Ad vector genome and VEGF protein levels were more than 1,000-fold and more than 90-fold higher, respectively, in this distribution than in other myocardial regions. In comparison, intracoronary injection yielded maximum myocardial Ad genome and VEGF levels 33-fold and 9-fold lower, respectively, than that after intramyocardial delivery. Angiograms obtained 28 days after intramyocardial AdGVVEGF121.10 demonstrated rapid circumflex reconstitution via collaterals localized to the region of vector administration.

Conclusions. These studies demonstrate that direct intramyocardial administration of AdGVVEGF121.10 results in focal genome and VEGF levels, including focal angiogenesis, sufficient to normalize blood flow to the ischemic myocardium, findings that are relevant to designing human trials of gene therapy-mediated cardiac angiogenesis.

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http://www.sciencedirect.com/science/article/B6TCH-4292G8K-6/2/b5ecf3c65d6d1abbb50f65e9b300196f

To identify changes in gene expression associated with emphysema, differential display was used to compare RNA extracted from emphysematous lung with that of unused donor tissue taken at the time of transplant. Two expressed clones with sequence homology to the 3' UTR of the murine flotillin-1 cDNA were identified. Flotillin-1 is a plasma membrane protein, which has been associated with detergent-insoluble glycolipid-rich domains and the formation of caveolae. One clone was 95 bp longer than the other. It arose from the use of a second polyadenylation signal and its existence was not due to differential expression nor to polymorphisms in the human flotillin-1 sequence. The 1839 bp human flotillin-1 sequence was completed by 5' RACE from a lung cDNA library. The human mRNA has a 1.9 kbase transcript being highly expressed in brain, heart and lung. The single copy flotillin-1 gene is located at 6p21.3 in the MHC class I region and consists of 13 exons over 15 kb. The ORF encodes a 427 residue protein with a molecular mass 47355 Da, and an isoelectric point 7.08. Human flotillin-1 has a 98% identity with the murine protein and a 47% identity with human flotillin-2. Flotillin-1 belongs to the Band 7.2/stomatin protein family, possessing a hydrophobic N-terminal region, predicted to form a single, outside to inside, transmembrane domain. The long central [alpha]-helical domain may form a coiled-coil. We have isolated and characterised a cDNA encoding the human flotillin-1 gene, which may play an important role in raft formation.

http://www.sciencedirect.com/science/article/B6TCH-3SY2FGJ-8/2/adcb58a73ac71b02143a990d991384e1

Chronic alcoholism causes a variety of ultrastructural, biochemical and functional alterations in the myocardium, but the underlying mechanisms are not well understood. Molecular changes that developed in the left ventricles of rats fed for 1 to 24 weeks on liquid diets containing ethanol as 36% of total calories were analyzed. Total tissue RNA and DNA were chemically extracted and measured by spectroscopic methods; mitochondrial DNA and mitochondrially-coded ribosomal RNA were measured at the 12s rRNA region by a quantitative polymerase chain reaction method; mitochondrial protein and enzyme activities were assayed. Ethanol-fed rats had 83.9+/-2.9% (mean+/-S.E.M.) as much DNA/g tissue and 74.7+/-3.9% as much total left ventricle DNA as pair-fed controls (PPP<0.001). Total left ventricle 12s rRNA was <40% of normal. There was little or no change in mitochondrial DNA levels measured at the 12s location. Mitochondrial cytochrome contents were reduced 26-38% in the ethanol-fed rats, but only after 24 weeks. This study shows that experimental alcoholism produces rapid and sustained decreases in left ventricle total RNA and DNA and mitochondrial ribosomal RNA. The observed effects would be expected to have a major impact on left ventricle structural integrity and functional capacity.


http://www.sciencedirect.com/science/article/B6TCH-3TYNMH7-F/2/6ba6f6f79b1359590d28038652d3f4a4

Lactoferrin is a mammalian iron-binding glycoprotein present in many biological secretions, such as milk, tears, semen and plasma and a major component of the specific granules of polymorphonuclear leucocytes. The effect of bovine lactoferrin (BLf) in apo-form or saturated with ferric, manganese or zinc ions, on human immunodeficiency virus type 1 (HIV-1) infection in the C8166 T-cell line was studied. Both HIV-1 replication and syncytium formation were efficiently inhibited, in a dose-dependent manner, by lactoferrins. BLf in apo and saturated forms markedly inhibited HIV-1 replication when added prior to HIV infection or during the virus adsorption step, thus suggesting a mechanism of action on the HIV binding to or entry into C8166 cells. Likewise, the addition of Fe3+BLf prior to HIV infection and during the attachment step resulted in a marked reduction of the HIV-1 DNA in C8166 cells 20 h after infection. The potent antiviral effect and the high selectivity index exhibited by BLf suggest for this protein, in apo or saturated forms, an important role in inhibiting the early HIV-cell interaction, even though a post adsorption effect cannot be ruled out.


Peroxisome proliferator-activated receptor [beta] (PPAR[beta]) is a member of the nuclear
hormone receptor superfamily and is a ligand activated transcription factor, although the precise genes that it regulates and its physiological and pathophysiological role remain unclear. In view of the association of PPAR[beta] with colon cancer and increased mRNA levels of PPAR[beta] in colon tumours we sought in this study to examine the expression of PPAR[beta] in human breast epithelial cells of tumorigenic (MCF-7 and MDA-MB-231) and non-tumorigenic origin (MCF-10A). Using quantitative RT-PCR we measured PPAR[beta] mRNA levels in MCF-7, MDA-MB-231 and MCF-10A cells at various stages in culture. After serum-deprivation, MDA-MB-231 and MCF-10A cells had a 4.2- and 3.8-fold statistically greater expression of PPAR[beta] compared with MCF-7 cells. The tumorigenic cell lines also exhibited a significantly greater level of PPAR[beta] mRNA after serum deprivation compared with subconfluence whereas such an effect was not observed in non-tumorigenic MCF-10A cells. The expression of PPAR[beta] was inducible upon exposure to the PPAR[beta] ligand bezafibrate. Our results suggest that unlike colon cancer, PPAR[beta] overexpression is not an inherent property of breast cancer cell lines. However, the dynamic changes in PPAR[beta] mRNA expression and the ability of PPAR[beta] in the MCF-7 cells to respond to ligand indicates that PPAR[beta] may play a role in mammary gland carcinogenesis through activation of downstream genes via endogenous fatty acid ligands or exogenous agonists.


http://www.sciencedirect.com/science/article/B6TCH-48NJ0G6-2/2/a7a9b75d95fd3414f4d1c2f59645e6d9

A novel, accurate, rapid and modestly labor-intensive method has been developed to quantitate specific mRNA species by reverse transcription-polymerase chain reaction (RT-PCR). This strategy combines the high degree of specificity of competitive PCR with the sensitivity of laser-induced fluorescence capillary electrophoresis (LIF-CE). The specific target mRNA and a mimic DNA fragment, used as an internal standard (IS), were co-amplified in a single reaction in which the same primers are used. The amount of mRNA was then quantitated by extrapolation from the standard curve generated with the internal standard. PCR primers were designed to amplify both a 185 bp fragment of the target cDNA for steroid 5[alpha]-reductase 1 (5[alpha]-R1) and a 192 bp fragment of the target cDNA for steroid 5[alpha]-reductase type 2 (5[alpha]-R2). The 5' forward primers were end-labeled with 6-carboxy-fluorescein (6-FAM). Two synthetic internal standard DNAs of 300 bp were synthesized from the sequence of plasmid pEGFP-C1. The ratio of fluorescence intensity between amplified products of the target cDNA (185 or 192 bp fragments) and the competitive DNA (300 bp fragment) was determined quantitatively after separation by capillary electrophoresis and fluorescence analysis. The accurate quantitation of low-abundance mRNAs by the present method allows low-level gene expression to be characterized.


http://www.sciencedirect.com/science/article/B6TCH-3VYXT6G-K/2/d8028985b1a3e515d543818207d133fb

Progastrin-derived peptides have been reported to stimulate mitogenesis in Swiss 3T3 fibroblasts [P. Singh, A. Owlia, R. Espeijo, B. Dai, Novel gastrin receptors mediate mitogenic effects of gastrin and processing intermediates of gastrin on Swiss 3T3 fibroblasts: Absence of detectable cholecystokinin (CCK)-A and CCK-B receptors. J. Biol. Chem. 270 (1995) 8429-8438]. The aim of
the present study was to determine the generality of these findings, by investigating the effect of endogenous and exogenous progastrin-derived peptides on the proliferation of the normal rat kidney fibroblast cell line NRK. Levels of endogenous progastrin-derived peptides were modified by stable transfection of NRK cells with tetracycline-repressible plasmids containing sequences encoding human gastrin in either the sense or antisense orientation. Expression of sense and antisense gastrin mRNA was demonstrated by reverse transcriptase PCR and by radioimmunoassay, and cell proliferation rates were determined by the colorimetric MTT assay. Sense clones produced full length human progastrin, but significant quantities of glycine-extended or amidated gastrin17 were not detected. Concentrations of endogenous rat progastrin in antisense clones were significantly lower than concentrations in clones transfected with vector only. However no difference in proliferation rate was observed between sense, antisense and vector-transfected clones. No stimulation of proliferation was observed in synchronised untransfected NRK cells after supplementation of media with gastrin17 or gastrin17gly in the concentration range 0.3 to 100 nM. Our results do not provide evidence in support of the hypothesis that endogenous or exogenous progastrin-derived peptides act as growth factors in NRK fibroblasts.


http://www.sciencedirect.com/science/article/B6VSG-417WC22-8/2/4955b9ff7078ef9f4a831f21c41e495a

Background: To determine whether genetic factors are involved in the development of renal dysfunction due to cyclosporine nephrotoxicity, we analyzed 2 polymorphisms in the signal sequence of the transforming growth factor (TGF)-[beta]1 gene; codon 10 (Leu10 -> Pro) and codon 25 (Arg25 -> Pro). Method Using sequence specific oligonucleotide probing, we analyzed both TGF-[beta]1 gene polymorphisms in cardiac allograft recipients (n = 168) who survived at least 1 year with minimal follow-up of 7 years. Patients received cyclosporine and steroids as maintenance immunosuppressive therapy. Renal dysfunction was defined as a serum creatinine >= 250 [mu]mol/liter. Results Renal dysfunction was observed in 2% (3/168) of the patients at 1 year, in 7% (11/160) at 3 years, in 12% (18/152) at 5 years, and in 20% (26/131) at 7 years post-transplantation. The genotypic distributions for TGF-[beta]1 codon 10 were 7% Pro/Pro, 61% Pro/Leu, and 32% Leu/Leu, and for codon 25 these percentages were 1% Pro/Pro, 12% Pro/Arg, and 87% Arg/Arg. We found an association between the TGF-[beta]1 genotype encoding proline at codon 10 and renal dysfunction. At 7 years post-transplantation, 26% (23/89) of the patients with the heterozygous Pro/Leu or homozygous Pro/Pro genotype had renal dysfunction vs only 7% (3/42) of the patients with the homozygous Leu/Leu genotype (p = 0.017). For the TGF-[beta]1 codon 25 genotypes, we found no association between TGF-[beta]1 genotypes and renal dysfunction. Conclusion Our data support the hypothesis that TGF-[beta]1 is involved in the process leading to renal insufficiency in cyclosporine-treated cardiac allograft recipients. In these patients the presence of TGF-[beta]1 Pro10 might be a risk factor.

Background: The regulatory cytokine transforming growth factor (TGF)-[beta]1 is thought to play a role in atherosclerotic heart disease as well as in idiopathic cardiomyopathy. The production of TGF-[beta]1 is genetically controlled as polymorphisms in the signaling sequence of the TGF-[beta]1 gene leucine10->proline and arginine25->proline are involved in the regulation of the protein production level. We investigated whether these polymorphisms are associated with end-stage heart failure caused by dilated cardiomyopathy (CMP) or ischemic heart disease (IHD).

Methods: We determined polymorphisms using sequence specific oligonucleotide probing (SSOP) in genomic DNA samples from heart transplant recipients (n = 253) and controls (n = 94). Indications for transplantation were dilated CMP (n = 109) and IHD (n = 144).

Results: We found a difference in TGF-[beta]1 codon 10 genotype distribution among patients with IHD, dilated CMP, and controls (p = 0.034; [chi]2 test). Patients with dilated CMP differed from patients with IHD (p = 0.044) and healthy controls (0.017). The genotype distribution between patients with IHD and controls was comparable. For codon 25, we found no difference in genotype distribution.

Conclusions: The Leu10->Pro (codon 10) polymorphism in the TGF-[beta]1 gene is associated with end-stage heart failure caused by dilated CMP and not with IHD. This observation suggests that TGF-[beta]1 is involved in the pathogenesis of CMP.


Background: In vitro, various cytokines can modulate the level of expression of major histocompatibility complex (MHC) Class II antigens. Major histocompatibility complex Class II hyperexpression occurs in many immunologic disorders in vivo, but the cytokines that affect this are difficult to analyze because they are produced in small amounts, they act locally, and their mRNAs have short half-lives.

Methods: We studied the expression of cytokines known to up-regulate MHC Class II genes in heart allografts in mice from B10.BR donors to B10.D2 recipients by reverse transcription of mRNA and polymerase chain reaction amplification. The I-A[beta]k gene expression was also studied in the same fully MHC incompatible strain combination.

Results: Messenger RNA for interferon (INF)-[gamma], interleukin (IL)-4, and tumor necrosis factor (TNF)-[alpha], known inducers of MHC Class II expression in vitro, could be detected in allografts either 24 hours before or simultaneously with massive induction of graft specific I-A[beta] mRNA. Interleukin-6 mRNA could be detected as early as 1 day after grafting.

Conclusions: These data suggest that known cytokine up-regulators of MHC Class II genes, i.e., IFN-[gamma], IL-4, and TNF-[alpha] may contribute to the upregulation of graft-specific MHC Class II antigens during an allograft reaction. Also, IL-6 expression in allografts may result from the stress of the grafting procedure, as it is evident very early after grafting.


http://www.sciencedirect.com/science/article/B6VSG-3X1W73Y-2/2/855fcb19c9c1419be534d2a36d3449267
Background: Preceding episodes of acute cellular rejection (ACR) may predispose lung allografts to the subsequent development of irreversible dysfunction or bronchiolitis obliterans syndrome (BOS). Other histologic patterns such as bronchiolitis obliterans with organizing pneumonia (BOOP), organizing pneumonia, lymphocytic bronchiolitis and diffuse alveolar damage (DAD) may also adversely affect allograft function. We have previously reported the predominant expression of Th1 cytokines (IL-2 and interferon [gamma]) in rejecting and Th2 (IL-10) in a tolerant model of rat lung transplantation. Here we correlate the "Th1/Th2 paradigm" in clinical lung transplantation with histologic findings and assess the effect on serial spirometric function.

Methods: We examined the mRNA expression of IL-2, interferon [gamma], IL-10 and ICAM-1 in 53 bronchoalveolar lavage (BAL) specimens from 23 lung transplant (LT) recipients utilizing qualitative "nested" reverse transcriptase polymerase chain reaction (RT-PCR). We also measured IgG1 and IgG2 levels in 44 BAL specimens by ELISA. The mRNA expression for cytokines, ICAM-1 and the IgG2/IgG1 ratios were correlated with the presence or absence of ACR and alternate "histologic patterns." Serial spirometry were analyzed for the 2-3 month interval before bronchoscopic (FOB) assessment to derive "baseline" forced expiratory volume-one second (FEV1) values. The change in FEV1 coincident with ([Delta]FEV1 pre) and for the 2-3 month interval subsequent to ([Delta]FEV1 post) FOB were expressed relative to "baseline" spirometric indexes.

Results: Detection of mRNA for interferon [gamma] and ICAM-1 correlated significantly with ACR, whereas IL-2 and IL-10 expression did not correlate. IL-10 was virtually "ubiquitous" in most BAL samples irrespective of the presence or absence of ACR. The highest correlation was observed with interferon [gamma] for acute cellular rejection whereupon the sensitivity was 77.7%, specificity 87.7%, positive predictive value 73.6% and negative predictive value 88.2%, although for ICAM-1 these values were 75%, 65.7%, 50.0% and 85.0%, respectively. Nevertheless, 4 of 5 episodes of respiratory tract infection (bacterial, CMV, Aspergillus spp.) were similarly associated with cytokine mRNA. The ratios of IgG2 to IgG1, a reflection of Th1/Th2 influence, were not statistically different when analyzed for the presence or absence of ACR (0.91 +/- 0.53 vs. 1.02 +/- 0.70, respectively; p = NS). By analysis of FEV1 trends, expression of interferon [gamma] was associated with a greater and persistent decrement ([Delta]FEV1 pre: -0.265 +/- 0.78 liters, and post: -0.236 +/- 0.1161; mean +/- SE) than ACR in the absence of interferon [gamma] expression (+0.158 +/- +0.065 and +0.236 +/- 0.007 liters, respectively) (Student-Newman-Keuls, p Conclusion: Our findings suggest that interferon [gamma] mRNA expression and ICAM-1 may be valuable in both the diagnosis and prognosis for lung allograft ACR. IL-10, a Th2 cytokine, was locally expressed both in the presence and absence of ACR. Expression of mRNA for interferon [gamma] in BAL and, to a lesser extent ICAM-1, were associated with increased lung allograft dysfunction. Whether BAL cytokine "immunosurveillance" would complement or possibly supplant a specific "histologic pattern" and thereby direct different therapies after lung transplantation, may be potentially rewarding areas of further investigation.

The Journal of Nutritional Biochemistry


http://www.sciencedirect.com/science/article/B6T8P-41FTT2P-5/2/110e9197ad6c6a08e73b979b0245dc5d

The metabolism of glutamine, the main respiratory fuel of enterocytes, is governed by the activity of glutaminase and glutamine synthetase. Because starvation induces intestinal atrophy, it might alter the rate of intestinal glutamine utilization. This study examined the effect of starvation on the activity, level of mRNA, and distribution of mRNA of glutaminase and glutamine synthetase in the
rat intestine. Rats were randomized into groups and were either: (1) fed for 2 days with rat food ad libitum or (2) starved for 2 days. Standardized segments of jejunum and ileum were removed for the estimation of enzyme activity, level of mRNA, and in situ hybridization analysis. The jejunum of the fed rats had a greater activity of both enzymes per centimeter of intestine (P < 0.01), a lower level of glutaminase mRNA, and a greater level of glutamine synthetase mRNA. In situ hybridization analysis showed that starvation does not alter the distribution of glutaminase and glutamine synthetase mRNA in the intestinal mucosa. This study confirms that starvation decreases the total intestinal activity per centimeter of both glutaminase and glutamine synthetase. More importantly, the results indicate that the intestine adapts to starvation by accumulating glutaminase mRNA. This process prepares the intestine for a restoration of intake.


http://www.sciencedirect.com/science/article/B6T8P-3S06DYB-B/2/79d610204eb2462ab5eedff6f4cf2575

Dietary methyl deficiency provides an ideal in vivo model system in which to study progressive alterations in DNA methylation patterns as they occur during multistage hepatocarcinogenesis. Weanling male F344 rats were given a semipurified diet deficient in the methyl-donors choline, methionine, and folic acid for a 36-week period with sampling intervals at 3, 9, 24, and 36 weeks. Using a genomic sequencing procedure based on the PCR amplification of bisulfite-modified DNA, the methylation status of individual CpG sites within exons 6 and 7 of the p53 gene in liver samples from control and deficient rats was assessed. Treatment of denatured nuclear DNA with sodium bisulfite converts unmethylated cytosine residues to uracil, which are then amplified as thymine in the PCR reaction. In contrast, methylated cytosines are resistant to bisulfite deamination under these reaction conditions and are amplified as cytosine. In this report, we describe a novel application of automated sequencing technology to estimate the proportion of methylated cytosines present at defined CpG sites within the total population of DNA molecules extracted. Using the bisulfite conversion-PCR genomic sequencing method, we demonstrate the validity of peak height analysis of co-eluting peaks in the autosequencer electrophoregram to estimate the percent methylation at a defined CpG site. The sensitivity of this method is demonstrated by the progressive loss of methyl groups at a defined CpG site in the methyl-deficient rats after 9, 24, and 36 weeks. The application of this sequence-specific technology will allow site-specific definition of the methylation status of each CpG site within a coding sequence or promoter region and should provide new insights into mechanisms and consequences of methylation dysregulation as a result of dietary deprivation of methyl donors.

The Journal of Pediatrics 1(1)


http://www.sciencedirect.com/science/article/B6WKR-4DP2SVY-1B/2/2455a541172253fb6ab6491d3bbc95c6
ObjectivesTo define the phenotype of congenital alveolar capillary dysplasia (ACD) as a first step toward mapping the responsible gene(s).

Study designAnalysis of pathology reports and microscopic slides of 23 subjects with ACD and sequence analysis of two candidate genes.

ResultsOur review of the pre- and postmortem records delineates both the natural history of this condition and the associated anomalies. Our collection of families corroborates the likely autosomal recessive nature of this condition in some families and provides additional data for genetic and prenatal counseling. Anomalies of many organ systems were detected either in the prenatal period or during the hospital course. However, some major anomalies were not detected until postmortem examination. Left-right asymmetry and gastrointestinal malrotation emerge as important, previously recognized but underappreciated phenotypic features of ACD. Finally, we used sequence analysis to exclude mutations in the coding region of two candidate genes, bone morphogenetic protein type II receptor (BMPR2) and endothelial monocyte-activating polypeptide II (EMAP II), as candidates for ACD.

ConclusionsUnderstanding the clinical spectrum of ACD and the cloning of an "ACD gene" both have implications for counseling, for prenatal testing, and for understanding the molecular pathophysiology of ACD and other organ malformations that are associated with this condition.

The Journal of Steroid Biochemistry and Molecular Biology (39)


http://www.sciencedirect.com/science/article/B6T8X-45VCGN6-3/2/ff22c9c64d988ae6c422c45a74de2352

Genetic factors play an important role in the pathogenesis of osteoporosis. The genes involved are, however, still largely unknown. In the present study, we have investigated whether sequence variations in the estrogen receptor beta (ER[beta]) gene are associated with bone mineral density (BMD) and biochemical markers of bone turnover in 79 Slovenian postmenopausal women with osteoporosis. We also assessed the response by BMD and bone markers to antiresorptive therapy with bisphosphonate alendronate. All eight exons of ER[beta] gene were amplified by polymerase chain reaction and screened for mutations by single-strand conformation polymorphism analysis. Potentially mutated samples were found only in exon 5 and sequence analysis identified the presence of a silent mutation in codon 328 with a nucleotide substitution GTG to GTA. For easier detection of this silent mutation, the RsaI restriction fragment length polymorphism analysis was developed. The frequencies of genotypes were as follows: Rr 5.1% and RR 94.9%. Between both genotypes, no significant differences in baseline lumbar spine and femoral neck BMD or in bone markers osteocalcin and deoxypyridinoline were observed. Similarly, no significant difference between RR and Rr genotypes in BMD or bone markers after 1 year of therapy was found. The increase in lumbar spine BMD after therapy was the only parameter that approached statistical significance (P=0.099). Patients with genotype Rr showed a smaller increase compared to those with RR. Our results suggest that RsaI polymorphism of ER[beta] gene is probably not an important genetic determinant of BMD and does not significantly influence the responsiveness to alendronate therapy.

insensitivity in breast cancer cells by co-treatment with histone deacetylation inhibitors." \(\textit{The Journal of Steroid Biochemistry and Molecular Biology}\) \textbf{89-90}: 245.

http://www.sciencedirect.com/science/article/B6T8X-4CDJGH7-4/2/3a97ff884a65e57f99c7aea7a4554e1

Proliferation of the non-malignant breast epithelial cell line, MCF-12A, is sensitively and completely inhibited by 1\(\alpha\),25-dihydroxyvitamin D3 (1\(\alpha\),25(OH)2D3) (ED90=70 nM). We used real time RT-PCR to demonstrate that the relative resistance to 1\(\alpha\),25(OH)2D3 of MDA-MB-231 cells (ED50>100 nM) correlated with significantly reduced Vitamin D receptor (VDR) and increased NCoR1 nuclear receptor co-repressor mRNA (0.1-fold reduction in VDR and 1.7-fold increase in NCoR1 relative to MCF-12A (P2D3 or potent analogs and the histone deacetylation inhibitor trichostatin A (TSA). For example, the co-treatment of 1,25-dihydroxy-16,23,Z-diene-26,27-hexafluoro-19-nor Vitamin D3 (RO-26-2198) (100 nM) plus TSA results in strong additive antiproliferative effects in MDA-MB-231 cells. This may represent novel chemotherapeutic regime for hormone insensitive breast cancer.


http://www.sciencedirect.com/science/article/B6T8X-3Y2N07Y-1F/2/43b70a5709cd41550108ed379f10b280

The estrogen receptor (ER) contains two transcriptional activation domains: AF-1 and AF-2. AF-2 is dependent on a highly species-conserved region of the ER. It has been shown that site-directed point mutations of conserved hydrophobic amino acids within this region reduce estrogen-dependent transcriptional activation. In addition, when these mutated ERs are transfected into HeLa cells, both tamoxifen and ICI 164,384 become strong agonists. The implication is that mutations in this region could account for the tamoxifen-stimulated tumors seen clinically. We performed single stranded conformational polymorphism (SSCP) analysis spanning the entire ER along with DNA sequencing of the AF-2 region of the ER isolated from two different tamoxifen-stimulated breast cancers, MCF-7/TAM and MCF-7/MT2, and a tamoxifen-stimulated endometrial cancer, EnCa 101. In addition, a tamoxifen-stimulated endometrial carcinoma cell line, the Ishikawa cell line, was also studied. There were no mutations found by SSCP analysis and sequencing of all four AF-2 regions also revealed no mutations. Mutations within the AF-2 region of the human ER do not appear to account for the growth of human breast and endometrial carcinomas that are used as reproducible laboratory models of tamoxifen-stimulated growth observed clinically.


http://www.sciencedirect.com/science/article/B6T8X-3Y2N07Y-1V/2/41d60d585b715d628090a439d3710f36

Mutations in the androgen receptor gene in 46,XY individuals can be associated with the androgen insensitivity syndrome, of which the phenotype can vary from a female phenotype to an underervilized or infertile male phenotype. We have studied the androgen receptor gene of androgen insensitivity patients to get information about amino acid residues or regions involved in DNA binding and transcription activation. Genomic DNA was analysed by PCR-SSCP under two
different conditions. Three new mutations were found in exon 1 of three patients with a female phenotype. A cytosine insertion at codon 42 resulted in a frameshift and consequently in the introduction of a premature stop at codon 171. Deletion of an adenine at codon 263 gave rise to a premature stop at codon 292. In both these cases, receptor protein was not detectable and hormone binding was not measurable. In a third patient, a guanine-to-adenine transition at codon 493 converted a tryptophan codon into a stop codon. Genital skin fibroblasts from this patient were not available. In exon 2 of the androgen receptor gene of a patient with receptor-positive androgen insensitivity, a cytosine-to-adenine transition, converting alanine 564 into an aspartic acid residue, resulted in defective DNA binding and transactivation. In three other receptor-positive androgen insensitivity patients no mutations were found with PCR-SSCP.


http://www.sciencedirect.com/science/article/B6T8X-4BYNMC3-1/2/6723940abedc140bf0747a536e6a9534

The use of chronic glucocorticoid (GC) therapy for the treatment of inflammatory diseases is limited by associated metabolic side effects, including muscle atrophy. Therefore, selective glucocorticoid receptor-(GR)-binding ligands that maintain anti-inflammatory activity and demonstrate diminished side-effect profiles would have great therapeutic utility. In this work, we use Taqman PCR and ELISA methods to show that GCs can inhibit basal, and lipopolysaccharide (LPS)-stimulated levels of cytokines IL-6 and TNF[alpha], and also the chemokine MCP-1 in a non-inflammatory system such as primary human skeletal muscle cells. In the murine C2C12 skeletal muscle cell line we observe a similar effect of GCs on IL-6 and MCP-1; however, in contrast to previous reports, we observe a time-dependent repression of TNF[alpha]. Furthermore, in skeletal muscle cells, concomitant with cytokine repression, GCs transcriptionally induce glutamine synthetase (GS), a marker for muscle wasting, in an LPS independent manner. Similarly, administration of dexamethasone to mice, previously administered LPS, results in an increase in GS and an inhibition of TNF[alpha] and MCP-1 in skeletal muscle tissue. Thus, skeletal muscle cells and tissues present a novel system for the identification of selective GR-binding ligands, which simultaneously inhibit cytokine expression in the absence of GS induction.


http://www.sciencedirect.com/science/article/B6T8X-3YYMR78-1G2/2/dc43f3901bd981fde8c87d0753c5ace6

Serum androgen-binding capacity in Djungarian hamsters, as in many other mammals, increases within days after birth and remains elevated until puberty. This increased activity has been attributed to a hepatic glycoprotein, sex hormone-binding globulin (SHBG), but expression of SHBG by the postnatal liver has not been demonstrated. Therefore, a full-length SHBG cDNA was cloned from the liver of neonatal hamsters and the expression of SHBG during development was examined. Hepatic SHBG RNA levels, as measured by both competitive RT-PCR and Northern analysis, were very low in fetal animals but increased significantly within 24 h of birth. Maximal values were maintained for 1 week after parturition, and then declined to basal adult levels. The developmental pattern in hepatic SHBG immunoreactivity, as determined by Western analysis, mirrored that of hepatic SHBG mRNA. However, changes in serum SHBG
immunoactivity and steroid-binding activity occurred approximately 1 week later. There were no sex differences in the levels of hepatic SHBG mRNA or protein during development, but serum immunoactivity tended to be higher in females at puberty. Sex- and age-related differences in the relative abundance of SHBG isoforms were also noted. Results of these studies demonstrate that Dzungarian hamsters express an authentic SHBG and indicate that the postnatal surge in serum androgen-binding activity is due to perinatal up-regulation of SHBG expression.


http://www.sciencedirect.com/science/article/B6T8X-42D80YH-3/2/3e7f3c2c5f29dc89ea8619d9e400b7b8

Estrogen receptor-mediated induction of zona radiata (ZR) and vitellogenin (VTG) mRNA and protein in rainbow trout (Oncorhynchus mykiss) was compared to assess their utility as biomarkers for exposure to estrogenic compounds. Partial sequences of rainbow trout ZR and [beta]-actin were cloned by reverse transcriptase polymerase chain reaction (RT-PCR) using degenerate primers based on conserved regions across a number of species. A 549 bp fragment of the rainbow trout ZR-gene showed a high degree of amino acid sequence identity to that of salmon (77%), winter flounder (64%), carp ZP2 (63%) and medaka (61%) ZR-proteins. The 1020 bp [beta]-actin fragment was approximately 100% identical to sequences from several species. Real-time PCR was used to quantify the induction of ZR-gene and VTG in rainbow trout liver after in vivo exposure to estradiol-17[beta] (E2) (0.01, 0.1, 1.0 or 10 mg/kg body weight (bw) fish) or [alpha]-zearalenol ([alpha]-ZEA) (0.1, 1.0 or 10 mg/kg bw). Real-time PCR and indirect enzyme-linked immunosorbent assay (ELISA) showed that ZR and VTG were induced in both the liver and the plasma after a single injection of E2 or [alpha]-ZEA. ZR was more responsive to low levels of E2 and [alpha]-ZEA than VTG, and real-time PCR was shown to be more sensitive than the ELISA. Rainbow trout ZR-gene and proteins provide a sensitive biomarker for assessing estrogenic activity.


http://www.sciencedirect.com/science/article/B6T8X-4757VHB-2/2/511d69b3eb04f78e0cb9554eeede8b8d2

The exact molecular mechanisms regulating estrogen receptor (ER)[alpha] expression in breast tumors are unclear, but studies suggest that the regulation is at least partly transcriptional. We therefore undertook a detailed analysis of ER[alpha] promoter activity in a number of breast cancer cell lines. We find that the majority of ER[alpha] promoter activity lies within the first 245 bp of the 5'-flanking region of the gene. Three elements essential for full ER[alpha] promoter transcriptional activity were identified within the -245 to -192 bp region in transient transactivation assays using linker-scanner mutation analysis. These three elements include two binding sites for the Sp1 family of transcription factors as well as a non-consensus E box. We show that both Sp1 and Sp3 bind to this region using electrophoretic mobility shift assays. Exogenous expression of Sp1 or Sp3 in Sp1/3-negative Drosophila Schneider SL2 cells results in transactivation of the -245 to +212 bp fragment of the ER[alpha] promoter. These data demonstrate that transcription of ER[alpha] is dependent upon the expression of members of the Sp1 family.
We applied the differential display RT-PCR (ddRT-PCR) technology to identify estrogen-regulated hepatic genes in the estrogen receptor expressing rat hepatoma cell line Fe33. Three genes of known sequences were detected by the ddRT-PCR approach: IGF binding protein-1 (IGFBP-1), vitamin D-dependent calcium-binding protein (CaBP9k) and major acute phase protein (MAP). Effects of ethinyl estradiol on the mRNA levels of these genes were confirmed by "Northern-blot" analysis. If given in combination with dexamethasone and glucagon, ethinyl estradiol caused 40-, 15- and 11-fold increases in the mRNA steady state level of IGFBP-1, CaBP9k and MAP, respectively, in Fe33 cells 24 h after addition of hormone. Besides ethinyl estradiol, the partial estrogen agonist OH-tamoxifen caused dose dependent effects on expression of MAP and IGFBP-1. Estrogen regulation of the respective genes and the modulatory effects of progesterone (10 mg/animal/day) were studied in ovariectomized rats treated subcutaneously for 14 days with 1 [mu]g/animal/day estradiol. "Northern-blot" analysis of liver RNA revealed a 6-fold stimulation of IGFBP-1 mRNA levels in estradiol-treated compared to vehicle-treated rats and a weak but detectable increase of MAP mRNA steady state level (1.6-fold) upon estradiol administration. No effect of estradiol treatment could be monitored for CaBP9k in rat liver. Modulatory effects of progesterone on estrogen-stimulated expression in the liver could be monitored for IGFBP-1 only. In an extension of our investigation on the expression of the three genes in rat liver, we determined their expression and hormonal regulation in the uterus of the same animals. In the uterus, estradiol caused an increase in CaBP9k mRNA. In contrast, IGFBP-1 mRNA levels increased dramatically upon progesterone administration, whereas no effect of estradiol treatment could be detected. MAP mRNA levels increased only after coadministration of estradiol and progesterone. In conclusion, the ddRT-PCR proved to be a powerful method to identify estrogen-regulated genes. The study on the hormonal regulation of three genes stimulated by estrogen in Fe33 cells revealed similarities and differences in their regulation in vivo and in vitro.

Acetoacetyl-CoA thiolase (ACoAT) catalyses the condensation of two acetyl-CoA molecules, the first step in the sterol biosynthetic pathway. We constructed a yeast strain containing a fusion of the promoter of the Saccharomyces cerevisiae ACoAT gene to a reporter gene (Escherichia coli [beta]-galactosidase). Reporter gene activity in this strain can be induced by a variety of inhibitors of sterol biosynthesis. These results suggest that the ACoAT gene is feedback regulated at the transcriptional level by products of the sterol biosynthetic pathway. The reporter gene approach described here may be used to screen chemical collections for compounds which inhibit fungal sterol biosynthesis.
The active form of Vitamin D, 1α,25-dihydroxyvitamin D3 [1,25-(OH)2D3], demonstrates potent antiproliferative actions on normal as well as on malignant cell types by blocking the transition from the G1- to the S-phase of the cell cycle. Key target genes for 1,25-(OH)2D3 in this non-classic effect remain largely unknown. Therefore, this study aims to identify genes that, through changes in expression after 1,25-(OH)2D3 treatment, contribute to the observed antiproliferative effect. cDNA microarrays containing 4600 genes were used to investigate changes in gene expression in MC3T3-E1 mouse osteoblasts at 6 and at 12 h after treatment with 1,25-(OH)2D3 (10⁻⁸ M), preceding (6 h) or coinciding with (12 h) the G1/S block in these cells. Approximately one fifth of the genes that were significantly down-regulated after a 12 h incubation period with 1,25-(OH)2D3 were genes involved in the DNA replication process, a basic process for cell growth that starts at the end of G1-phase and continues in S-phase. Down-regulation of these genes by 1,25-(OH)2D3 was confirmed by quantitative RT-PCR in MC3T3-E1. In conclusion, cDNA microarrays revealed that treatment of MC3T3-E1 cells with 1,25-(OH)2D3 resulted in the down-regulation of DNA replication genes in parallel with the observed G1/S-arrest.


The recent observation that estrogen synthesis occurs in osteoblast-like cells has suggested the aromatase activity as a possible local modulator of bone remodeling in post-menopausal women. To provide further insights into the androstenedione conversion to estrogen in bone-derived cells, we examined the human leukaemic cell line FLG 29.1, which is induced to differentiate toward the osteoclastic phenotype by TPA and TGF-β1. Southern blot of RT-PCR products with a 32P-labeled cDNA probe for the human aromatase demonstrated that FLG 29.1 cells express aromatase mRNA. The enzyme activity, determined by measuring [3H]H2O release from [3H]androstenedione, obeyed Michaelis-Menten kinetic with apparent Km and Vmax values ranging from 5 to 10 nM and from 200 to 400 fmol/mg protein/6 h. Gene expression, enzyme activity and protein immunoreactivity, evaluated by immunocytochemistry, were stimulated in a time-dependent fashion by 5% charcoal-stripped FCS and by either 1-100 nM TPA or 0.01-0.5 ng/ml TGF-β1, with maximal responses after 2-3 h exposure. After 24 h incubation of FLG 29.1 cells in the absence of these stimuli the aromatase mRNA and the protein were barely detectable. These findings demonstrate that cells of the osteoclastic lineage synthesize estrogen in vitro and that local cytokines, such as TGF-β1, are able to induce androstenedione conversion.


Sequential hermaphroditism is a common reproductive strategy in many teleosts. Steroid production is known to mediate both the natural and induced sex change, yet beyond this the physiology directing this process has received little attention. Cytochrome P450 aromatase is a key enzyme in the hormonal pathway catalysing the conversion of sex steroids, androgens to oestrogens, and thus is highly relevant to the process of sex change. This study reports the
isolation of cDNA sequences for aromatase isoforms CYP19A1 and CYP19A2 from teleost species representing three forms of sexual hermaphroditism: Lates calcarifer (protandry), Cromileptes altivelis (protogyny), and Gobiodon histrio (bi-directional). Deduced amino acid analysis of these isoforms with other reported isoforms from gonochoristic (single sex) teleosts revealed 56-95% identity within the same isoform while only 48-65% identity between isoforms irrespective of species and sexual strategy. Phylogenetic analysis supported this result separating sequences into isoform exclusive clades in spite of species apparent evolutionary distance. Furthermore, this study isolates 5' flanking regions of all above genes and describes putative cis-acting elements therein. Elements identified include steroidogenic factor 1 binding site (SF-1), oestrogen response element (ERE), progesterone response element (PRE), androgen response element (ARE), glucocorticoid response elements (GRE), peroxisome proliferator-activated receptor [alpha]/retinoid X receptor [alpha] heterodimer responsive element (PPAR[alpha]/RXR[alpha]), nuclear factor kappa[beta] (NF-kappa[beta]), SOX 5, SOX 9, and Wilms tumor suppressor (WTI). A hypothetical in vivo model was constructed for both isoforms highlighting potential roles of these putative cis-acting elements with reference to normal function and sexual hermaphroditism.


http://www.sciencedirect.com/science/article/B6T8X-47425F0-1/2/067b566ec5d6852cbf157df2ea424189

Aromatase, the product of the CYP19 gene, plays a key role in androgenic steroids transformation into estrogens from various hormonal sensitive tissues. Thus, in situ expression of CYP19 has been suggested to be involved in breast tumor growth especially in post-menopausal patients. We developed a real-time quantitative RT-PCR assay based on fluorescent TaqMan(R) methodology to quantify total CYP19 gene expression at the mRNA level in breast tumors. This method, based on nucleic acid quantification in homogeneous solutions, has the potential to become a standard in terms of its sensitivity, wide dynamic range and high-throughput capacity. In a well-defined series of 107 post-menopausal breast tumor samples, relative CYP19 mRNA levels ranged from 1 to 131. Among the four major CYP19 exon I-spliced transcripts, designated I.a, I.b, I.c and I.d, mRNA levels of the latter three correlated positively with total CYP19 mRNA levels. In ER[alpha]-positive breast tumors, CYP19 and ER[alpha] mRNA levels correlated negatively with each other (P=0.0078, r=-0.266), while CYP19 and ER[beta] mRNA levels correlated positively (P=0.00012, r=0.388). Patients with high CYP19 mRNA levels did not relapse more frequently or have shorter relapse-free survival than other patients. Finally, mRNA levels of IL6, a major CYP19 regulatory factor, were significantly higher in tumors strongly expressing CYP19 than in tumors weakly expressing CYP19 (P=0.018). In conclusion, CYP19 expression did not influence the outcome of post-menopausal patients with breast cancer.


http://www.sciencedirect.com/science/article/B6T8X-4CDJGF1-1/2/56f56ffbaafe864273915528130e0929

Normal prostate epithelial cells are acutely sensitive to the antiproliferative action of 1[alpha],25-dihydroxyvitamin D3 (1[alpha],25(OH)2D3), whilst prostate cancer cell lines and primary cultures display a range of sensitivities. We hypothesised that key antiproliferative target genes of the
Vitamin D receptor (VDR) were repressed by an epigenetic mechanism in 1[alpha],25(OH)2D3-insensitive cells. Supportively, we found elevated nuclear receptor co-repressor and reduced VDR expression correlated with reduced sensitivity to the antiproliferative action of 1[alpha],25(OH)2D3. Furthermore, the growth suppressive actions of 1[alpha],25(OH)2D3 can be restored by co-treatment with low doses of histone deacetylation inhibitors, such as trichostatin A (TSA) to induce apoptosis. Examination of the regulation of VDR target genes revealed that co-treatment of 1[alpha],25(OH)2D3 plus TSA co-operatively upregulated GADD45[alpha]. Similarly in a primary cancer cell culture, the regulation of appeared GADD45[alpha] repressed. These data demonstrate that prostate cancer cells utilise a mechanism involving deacetylation to suppress the responsiveness of VDR target genes and thus ablate the antiproliferative action of 1[alpha],25(OH)2D3.


http://www.sciencedirect.com/science/article/B6T8X-47PR5MP-1F1/2/9771df3e80ee71dc7828db339f9269ba

Placental aromatase deficiency, which was characterized by maternal and fetal virilization and by a low level of estrogen excretion into urine during pregnancy, was studied by biochemical and molecular genetical techniques. Among enzymes participating in the electron transport system of the patient's placental microsomes, only aromatase activity was observed to be reduced (< 3% of normal levels). Northern and Western blotting analyses showed that the transcription of the aromatase gene and the translation of its mRNA seemed to proceed normally in the patient's tissue. However, the aromatase cDNA isolated from the patient was found to contain an extra DNA fragment of 87 base pairs (bp) which encoded 29 amino acids in frame but no termination codon. The insertion was located at the splicing point between exon 6 and intron 6 of the normal aromatase gene. The extra DNA fragment represented the first part of intron 6 except that its initial GT was altered to GC. These findings indicated that, in the patient's aromatase gene, the splicing between exon 6 and intron 6 did not occur at the normal position. This reflected the presence of one point mutation in its consensus sequence which caused the next cryptic consensus sequence 87 bp downstream, to be used according to the canonical GT/AG rule. The protein molecule thus translated contained an extra 29 amino acids. Furthermore, the patient's aromatase cDNA was observed to produce a protein molecule with a trace of activity in the transient expression system of COS-7 cells and in the high level expression system of baculovirus-insect cells. Direct DNA sequencing of aromatase genes from the patient and parents confirmed that this deficiency is a hereditary disease with an autosomal recessive inheritance pattern. The patient and parents are homozygote and heterozygotes, respectively, for this mutation.


http://www.sciencedirect.com/science/article/B6T8X-45X2KX9-6/2/906e80990a586772512402b2f054813

The antiproliferative effect of 1[alpha],25-dihydroxyvitamin D3 (1[alpha],25(OH)2D3) has been studied for a decade in diverse model systems, but the signalling pathways linking 1[alpha],25(OH)2D3 to cell cycle arrest remains unclear. In our attempt to establish a model system which would allow further identification of important players in the process of the 1[alpha],25(OH)2D3 imposed cell cycle arrest, we have isolated derivatives of the human breast
cancer cell line MCF-7 and chosen two nearly 1α,25(OH)2D3 resistant and two hypersensitive sub-clones. Investigation of cell cycle proteins regulated by 1α,25(OH)2D3 in these clones indicates that activation of one component/pathway is responsible for the linkage between 1α,25(OH)2D3 and growth arrest. Protein levels of the Vitamin D receptor (VDR) were elevated in sensitive cells upon 1α,25(OH)2D3 treatment, whereas resistant clones were unable to induce VDR upon 1α,25(OH)2D3 treatment. Our data show that VDR protein levels and the ability of a cell to induce VDR upon 1α,25(OH)2D3 treatment correlate with the antiproliferative effects of 1α,25(OH)2D3, and suggest that the level of VDR in cancer cells might serve as a prognostic marker for treatment of cancer with 1α,25(OH)2D3 analogues.


The gene encoding steroid 21-hydroxylase activity, P450c21B, is located in the major histocompatibility complex (MHC) class III region, in close proximity to a highly homologous pseudogene, P450c21A. Recombinations between P450c21B and P450c21A have been shown to result in deficiency of 21-hydroxylase activity, the usual cause of congenital adrenal hyperplasia (CAH). A mutant P450c21 gene from a patient with simple virilizing CAH was identified and shown to be consistent with a recombination between P450c21A and P450c21B. Sequence analysis of the mutant gene showed the recombination site to be located between the first exon and the second intron. The mutant gene encodes a leucine instead of the normal proline at codon 31. This mutation resides on a chromosome bearing the HLA-B44 serotype. A comparison of mutation associated with HLA-B44 and that normally found with the HLA-Bw47 serotype suggests that the HLA-B44 mutations are of more ancient origin. The patient's homologous chromosome has a deletion of P450c21B. Endocrinological testing therefore allows for testing of the mutant gene in genetic isolation. Such testing demonstrated that the patient was capable of producing aldosterone and retaining sodium in response to a low-sodium diet, indicating that the mutant gene encodes an enzyme with partial 21-hydroxylase activity.


http://www.sciencedirect.com/science/article/B6T8X-3YXC0B3-1W/2/d6d67e1306f3fc93a4c25c03ffe7c5b2

Steroid 21-hydroxylase activity was assayed in low-speed supernatants prepared from whole cell homogenates of mouse and rat tissues. Kidney supernatants had an activity which was approximately 2-5% that of adrenal preparations while heart muscle was found to be without 21-hydroxylase activity. When the enzyme kinetics were characterized, both adrenal and kidney low-speed supernatants demonstrated saturation kinetics, but with very different Vmax and Km values. Using polymerase chain reaction amplification after reverse transcriptase synthesis of cDNA from isolated RNA (RT-PCR), we found low levels of mRNA for steroid 21-hydroxylase in mouse kidney, but none in heart muscle. Thus, extra-adrenal steroid 21-hydroxylase activity in the kidney may be mediated by the same enzyme as found in adrenals.

The structure and expression of a clone containing the promoter region, all of exon 1, and part of the first intron of the human mineralocorticoid receptor (hMR) gene is presented. The clone has three sets of CAAT and TATA elements, one located at the very 5'-end of the clone, one located just 5'- to the start of transcription, and one set located in intron A, approximately 300 bp into the intron. The major start of transcription site by primer extension analysis and ribonuclease protection assays is located 26 bp downstream of a TATA-like box (TTTAA) and 90 and 143 bp downstream, respectively, of two CCAAT boxes. Putative cis-transcription factor binding sites are as follows: two potential AP1 sites, one potential AP2 site, two ATF/CREB sites, six potential GC boxes or SP1 sites, one potential perfect half-palindromic estrogen response element, and three potential PEA3 sites. Therefore, the hMR promoter region contains elements characteristic of both regulated genes and "housekeeping" genes. CAT assays of overlapping deletions of the promoter region demonstrated tissue-specific regulation in human neuroepithelioma (SK-N-MC-IXC) and non-neuronal, peripheral choriocarcinoma cell lines (JEG-3).


We have demonstrated the expression of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA in human uterine endometrium, using the reverse transcription-polymerase chain reaction-Southern blot and DNA sequencing analyses. Analysis of the missing base pairs corresponded to the entire exon VII, which are considered to encode a portion of the steroid-binding site. Therefore, the steroid-binding affinity of this variant might be different from that of the SHBG wild type. In uterine endometria, the wild-type and variant mRNA levels tended to increase with the advance of the menstrual phase, but the ratio of the SHBG variant mRNA to SHBG wild-type mRNA levels showed no significant difference during the menstrual cycle. So far, there are no indications that the SHBG variant has any biological or clinical implications in human uterine endometrium.


This study was designed to determine the clinical implications of intracellular expression of sex hormone-binding globulin (SHBG) wild-type and exon 7 splicing variant mRNAs in secondary spreading lesions of gynecologic cancers using the reverse transcription-polymerase chain
reaction-Southern blot and DNA sequencing analyses. Compared with primary cancers, a relative increase in SHBG variant mRNA to wild-type mRNA expression was observed (4/10 cases of uterine endometrial cancers, 5/10 cases of uterine cervical cancers, 6/10 cases of ovarian cancers) or the expression of SHBG wild-type and variant mRNAs could not be detected (5/10 cases of uterine endometrial cancers, 3/10 cases of uterine cervical cancers, 4/10 cases of ovarian cancers). On the other hand, alteration to a relative increase in SHBG wild-type mRNA expression in the metastatic lesions occurred in only 3 cases (1/10 cases of uterine endometrial cancers and 2/10 cases of uterine cervical cancers) analyzed. These results suggest that the transcription of SHBG mRNA and the regulation of its splicing might be altered with metastatic potential, and this status might be involved in a change in steroidal dependency of metastatic lesions.


http://www.sciencedirect.com/science/article/B6T8X-3S12BV0-9/2/395bd3c3d1b50b244f424636731df568

To ascertain one of the biological effects of danazol and progesterone on the uterine endometrial cancer cell line, Ishikawa, we investigated the effects of these steroids on sex hormone-binding globulin (SHBG) mRNA expression by competitive reverse transcription-polymerase chain reaction-Southern blot analysis (RT-PCR-SBA). Estradiol-17[beta] (E2) in any concentration given did not exert any significant effect on the expression of SHBG mRNA. Danazol and progesterone significantly (P < 10^-6 and 10^-8 M, respectively. Progesterone, in a low concentration (10-10 M) with E2 (10-8 M), significantly (P < 10^-6 to 10^-5 M) with E2 (10-8 M) significantly (P < 10^-6 M) with or without E2 (10-8 M), except for a temporal increase by progesterone. These findings suggest that danazol and progesterone in a superphysiological milieu down-regulate the intracellular SHBG-related steroidal actions, and that progesterone in a physiological milieu with estrogen up-regulates it in a hormone-dependent cell line. A decrease of intracellular SHBG caused by high-dose danazol or progesterone might partly contribute to the abolition of the intracellular estrogen-dominant milieu, and be related to the inhibition of estrogen-dependent growth of some endometrial cancer cells.


http://www.sciencedirect.com/science/article/B6T8X-3S3MPFK-D/2/a9003c5634beb0b247abe15737de2db9

We have explored the mechanism of estrogen-induced growth in human uterine leiomyomas from the aspect of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA expression using the reverse transcription-polymerase chain reaction-Southern blot and DNA sequencing analyses. The results were obtained by analysis of the missing base pairs corresponding to the entire exon VII, which are considered to encode a portion of the steroid-binding site. This absence replaces 118 amino acids from the carboxy-terminus of SHBG with nine different amino acid residues due to the formation of a new stop codon at residue 334. The ratio of the SHBG variant to its wild-type mRNA levels in uterine leiomyomas was reduced, compared with that in the corresponding myometria in individual cases, while the SHBG wild-type and variant mRNA levels showed no significant difference during the menstrual phase. These studies demonstrate coexpression of SHBG exon VII splicing variant mRNA with its wild-type in human uterine
myometria and leiomyomas. The reduced expression of the SHBG variant to wild-type mRNA levels in leiomyoma might be involved in the intracellular estrogen-predominant milieu, plausibly assisting in the development and growth of the leiomyoma.


http://www.sciencedirect.com/science/article/B6T8X-3YXC07S-2/2/6fe7afe91d7a0106d0443ba4cb6cd7c

To more fully understand the role of sex hormone-binding globulin (SHBG) on the intracellular steroidal action in endometrial cancers, we investigated the expression of SHBG mRNA as the substitute of SHBG expression in human endometrial cancers. In the present study, the levels of SHBG mRNA were analyzed using competitive reverse transcription-polymerase chain reaction (RT-PCR)-Southern-bolt analysis. The higher level of SHBG mRNA tended to be expressed in the normal secretory and late proliferative phase endometrium > early proliferative phase endometrium > well differentiated adenocarcinoma of the endometrium (G1) > moderately differentiated adenocarcinoma (G2) > poorly differentiated adenocarcinoma (G3), in the order shown. These studies indicate that endometrial cancer cells might synthesize intracellular SHBG to conserve their estrogen-dependent properties. Further, it indicates that endometrial cancer cell synthesis of SHBG mRNA is lost as these cells undergo de-differentiation.


http://www.sciencedirect.com/science/article/B6T8X-42D80YH-2/2/7aeaa7cb79cddb57e664a9fa8f6fc00

Recent studies point to a key role for the estrogen synthesizing enzyme P450 aromatase (P450 arom) in ovary determination in fish, birds and reptiles. It is unclear whether estrogen synthesis is important in sex determination of Xenopus gonad. To determine whether the aromatase gene is transcribed in the gonads of Xenopus tadpoles during the sex determination, we cloned a P450 arom cDNA and examined the level of P450 arom and estrogen receptor (ER) gene expression in association with estrogen activity. cDNA clones for P450 arom were isolated from a Xenopus ovarian cDNA library. There was an open reading frame (ORF) of 1500 bp from the ATG start to TAA stop codons encoding 500 predicted amino acids. cDNAs for P450 arom have previously been cloned from various vertebrates. The homology between the Xenopus P450 aromatase and the human P450 arom was higher. The expression of the P450 arom gene was mainly limited to reproductive organs. To determine the beginning of estrogen activity in gonads of embryos, expression of the aromatase and ER gene was also examined by RQ-RT-PCR. Both Xenopus aromatase and ER mRNA was detected at stage 51 in gonads. These observations are consistent with estrogens having a key role in ovarian development in various other vertebrates.


http://www.sciencedirect.com/science/article/B6T8X-3YYT600-
The cDNA coding for pig testicular 3[alpha]/[beta] (20[beta])-hydroxysteroid dehydrogenase was expressed in Escherichia coli by placing it under the control of an isopropylthiogalactoside (IPTG) inducible tac promoter. Production of 3[alpha]/[beta] (20[beta])-HSD was demonstrated by Western blotting and by catalytic activity with 5[alpha]-dihydrotestosterone as a substrate for 3[alpha]/[beta]-HSD, and progesterone and 17[alpha]-hydroxyprogesterone as substrates for 20[beta]-HSD in the presence of NADPH. The 3[alpha]/[beta] (20[beta])-HSD enzyme was detected in a soluble fraction of the lysate of E. coli added to IPTG to induce the synthesis of the protein. Its molecular weight was estimated to be 30.5 kDa by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Recombinant 3[alpha]/[beta] (20[beta])-HSD was purified to apparent homogeneity as determined by SDS-PAGE by column chromatography using DEAE-cellulose. The purified enzyme reduced not only steroids but also prostaglandins and other carbonyl compounds including aldehydes, ketones and quinones as demonstrated in native enzymes purified from pig testes. The amino terminus of the purified enzyme was serine which was coded next to the ATG start codon, and the sequence of the amino terminal 24 residues was identical with the coding amino acid in the cDNA; whereas, the amino terminus of the native 3[alpha]/[beta] (20[beta])-HSD was not detected suggesting that the N-terminal amino acid was blocked.


Calcitriol, a hormonal form of Vitamin D, regulates growth of normal and cancer cells of various origins by modulation of peptide growth factors signaling. Platelet-Derived Growth Factor (PDGF) signaling pathway is involved in prostate cancer progression. We studied the expression of PDGF receptors in human prostate primary stromal cells and cancer epithelial cell lines and growth response to PDGF-BB isoform. We found that the expression of PDGF receptors and PDGF-BB-mediated cell growth are regulated by calcitriol in prostate cells. Quantitative RT-PCR analysis revealed a lower level of mRNA for PDGF receptors in LNCaP and PC-3 cells than in primary stromal cells. Western blotting showed a high amount of PDGFR[alpha] and [beta] proteins in primary stromal cells that could not be detected in LNCaP, which may explain the resistance of LNCaP cells to growth-promoting effect of PDGF-BB. Addition of Epidermal Growth Factor (EGF) to the culture medium induces the expression of PDGFR[beta] and restores responsiveness of LNCaP to PDGF-BB to some extent. Calcitriol down-regulates PDGFR[beta] expression and negatively regulates PDGF-mediated cell growth. Calcitriol does not affect PDGFR[alpha] and PDGF-B mRNA expression. We suggest that inhibition of PDGFR[beta] expression by calcitriol might reduce responsiveness of prostate cells to mitogenic action of PDGF-BB.


http://www.sciencedirect.com/science/article/B6T8X-4CSG31N-1/2/c9bd1e04cbe29676a9799dd33289d75

In this study, the methylation status of the distal promoter F of estrogen receptor alfa (ER[alpha]) gene in human osteoblastic cells was investigated. The activity of this promoter is responsible for the ER[alpha] gene transcription in bone tissue. The methylation status of promoter F was here evaluated, for the first time, by direct sequencing of bisulfite-treated genomic DNA, at 10 CpG
specific sites localized in a region of about 800 bp. An heterogeneous methylation pattern was observed. The most notable difference was found at four particular CpGs, distant from the exon F transcription start site, showing a methylation status that correlates with the expression level, being ER[alpha] mRNA transcription reduced in a partially methylated cells but preserved in demethylated cells. The other CpG sites, localized around the transcription start site, were always demethylated except for MG-63 cells showing the lowest level of ER[alpha] expression. By quantitative RT-PCR analysis we demonstrated that ER[alpha] gene expression was higher in primary osteoblasts than in bone-derived cells (MG-63 and SaOS-2) and in all cases the ER[alpha] mRNA is represented by the isoform F. The same 10 CpG sites were investigated in non-osseous cell lines and were found fully methylated in ER[alpha]-negative breast cancer cells (MDA-MB-231) and completely demethylated in ER[alpha]-positive breast cancer cells (MCF7). The overall results suggest that methylation of the CpG sites inside ER[alpha] gene promoter F here analyzed may contribute to ER[alpha] transcriptional control, directly or indirectly, influencing the tissue specific expression of the gene.


http://www.sciencedirect.com/science/article/B6T8X-408KCH3-72f9006e6d04d66f9f263e72cea05ecf46

Estrogen receptor (ER) alpha splice variant transcript profiles were analyzed by RT-PCR in six ER positive breast cancer cell lines, MCF-7, T47D, ZR-75, LCC1, LCC2 and LCC9, three ER negative cell lines, MDA-MB-435, MDA-MB-235 and LCC6, and three ER positive malignant breast tumors using targeted primers which specifically anneal to the splice junctions of exon 2[Delta], exon 3[Delta], exons 2-3[Delta], exon 4[Delta], exon 5[Delta], exon 6[Delta] and exon 7[Delta]. The partner primers were chosen such that largest possible transcripts were amplified between exons 1 and 8. The results described here show that each splice specific primer amplified not only the single exon deleted transcript but also a number of related transcripts that have deletions in various combinations of exons. The exon 2[Delta] specific primer amplified five transcripts that have deletions in exon 2, exons 2 and 7, exons 2, 5, and 7, exons 2 and 4-5, and exons 2 and 4-6. The exon 3[Delta] specific primer amplified two transcripts that have deletions in exon 3, and exons 3 and 7. The exon 2-3[Delta] specific primer amplified three products that have deletions in exons 2-3, exons 2-3 and 7 and exons 2-3, 5 and 7. The exon 4[Delta] specific primer amplified two products that have deletions in exon 4, and exons 4 and 7. The exon 5[Delta] specific primer amplified three transcripts, that have deletions in exon 5, exons 5 and 2, and exons 5 and 2-3. The 6[Delta] specific primer amplified only one transcript that has a deletion in exon 6. The 7[Delta] specific primer amplified four transcripts, that have deletions in exon 7, exons 7 and 4, exons 7 and 3-4, and exons 7 and 3-5. None of the above splice specific primers amplified the wild type ER sequences. The six ER positive cell lines differed in the patterns of the variant transcripts and among the three ER negative cell lines analyzed, only MDA-MB-435 showed the presence of exon 2[Delta] and exon 4[Delta] transcripts. Analyses in the tumor samples indicated that the above transcripts are extensively modified.


http://www.sciencedirect.com/science/article/B6T8X-49505JV-1/2/04498f1f887509bfbf19b05a8d551d051
Transcriptional cross-talk exists between the estrogen receptor (ER[alpha]) and retinoic acid receptor (RAR) pathways in human breast cancer cells. We have previously shown that re-expression of ER[alpha] in ER-negative cells stimulates the transcriptional and growth inhibitory effects of all-trans-retinoic acid (tRA) by a mechanism that is independent of the ER ligands estradiol and tamoxifen. In this study, we generated cell lines stably expressing ER[alpha]-deletion mutants to elucidate the mechanism whereby ER[alpha] modulates RAR transcriptional activity. Using RT-PCR and RNAse protection assays, we observed that expression of ER[alpha] suppresses basal expression of the RA-responsive gene RAR[beta]2, while allowing it to be strongly induced by tRA. Repression of basal RAR[beta]2 transcription was confirmed by transient expression of the reporter plasmid [beta]RE-tk-CAT, containing the RAR[beta]2 promoter. In the ER[alpha]-negative cells, on the other hand, transcription was only weakly induced by RA. We further determined that this effect of ER[alpha] on RAR[beta]2 induction required the N-terminal AF-1-containing region, including the DNA-binding domain, but was independent of the C-terminal ligand-binding domain. Consistent with these results, the ER agonist estradiol and the AF-2 antagonist 4-hydroxytamoxifen had no significant effect on [beta]RARE activity. Conversely, the full ER antagonist ICI 182,780, which blocks ER[alpha] AF-1 activity, was able to completely relieve repression of basal [beta]RARE activity. The effect of ER[alpha] is specific for RAR-mediated transcription and does not occur on promoters containing typical response elements for the Vitamin D or thyroid hormone receptors. Moreover, the cross-talk between ER[alpha] and RAR does not seem to be mediated by sequestration of a number of common co-regulators, suggesting a novel mechanism whereby the N-terminal region of ER[alpha] modulates the transcriptional activity of RAR.


http://www.sciencedirect.com/science/article/B6T8X-47DTCF8-35/2/96352fc39449c7515e900f26cb5a07bc

The 5' end of the steroid 21-hydroxylase B gene encompassing putative control regions and the first 3 exons, has been selectively amplified in vitro from a number of patients with congenital adrenal hyperplasia caused by a deficiency of this enzyme. Sequence analysis has revealed a number of isolated instances of gene conversion to the 21-hydroxylase A sequence. One mutation, a C to G transversion at the 3' end of the second intron, thought to lead to incorrect splicing of the mRNA, was found in 11 subjects all with the classical form of the disease.


http://www.sciencedirect.com/science/article/B6T8X-49J8SJY-7Z/2/de566f8c056ea881704b39d442c9ec1

Estrogens play a crucial role in the development and evolution of human breast cancer. However, it is still unclear whether estrogens are carcinogenic to the human breast. There are three mechanisms that have been considered to be responsible for the carcinogenicity of estrogens: receptor-mediated hormonal activity, a cytochrome P450 (CYP)-mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates, and the induction of aneuploidy by estrogen. To fully demonstrate that estrogens are carcinogenic in the human breast through one or more of the mechanisms explained above it will require an experimental system in which, estrogens by itself or one of the metabolites would induce transformation phenotypes indicative of
neoplasia in HBEC in vitro and also induce genomic alterations similar to those observed in spontaneous malignancies. In order to mimic the intermittent exposure of HBEC to endogenous estrogens, MCF-10F cells that are ER[alpha] negative and ER[beta] positive were first treated with 0, 0.007, 70 nM and 1 [mu]M of 17[beta]-estradiol (E2), diethylstilbestrol (DES), benz(a)pyrene (BP), progesterone (P), 2-OH-E2, 4-hydroxy estradiol (4-OH-E2) and 16-[alpha]-OH-E2 at 72 h and 120 h post-plating. Treatment of HBEC with physiological doses of E2, 2-OH-E2, 4-OH-E2 induce anchorage independent growth, colony formation in agar methocel, and reduced ductulogenic capacity in collagen gel, all phenotypes whose expression are indicative of neoplastic transformation, and that are induced by BP under the same culture conditions. The presence of ER[beta] is the pathway used by E2 to induce colony formation in agar methocel and loss of ductulogenic in collagen gel. This is supported by the fact that either tamoxifen or the pure antiestrogen ICI-182,780 (ICI) abrogated these phenotypes. However, the invasion phenotype, an important marker of tumorigenesis is not modified when the cells are treated in presence of tamoxifen or ICI, suggesting that other pathways may be involved. Although we cannot rule out the possibility, that 4-OH-E2 may interact with other receptors still not identified, with the data presently available the direct effect of 4-OH-E2 support the concept that metabolic activation of estrogens mediated by various cytochrome P450 complexes, generating through this pathway reactive intermediates that elicit direct genotoxic effects leading to transformation. This assumption was confirmed when we found that all the transformation phenotypes induced by 4-OH-E2 were not abrogated when this compound was used in presence of the pure antiestrogen ICI. The novelty of these observations lies in the role of ER[beta] in transformation and that this pathway can successfully bypassed by the estrogen metabolite 4-OH-E2. Genomic DNA was analyzed for the detection of micro-satellite DNA polymorphism using 64 markers covering chromosomes (chr) 3, 11, 13 and 17. We have detected loss of heterozygosity (LOH) in chr13q12.2-12.3 (D13S893) and in chr17q21 (D17S800) in E2, 2-OH-E2, 4-OH-E2, E2 + ICI, E2 + tamoxifen and BP-treated cells. LOH in chr17q21.1-21.2 (D17S806) was also observed in E2, 4-OH-E2, E2+ICI, E2+tamoxifen and BP-treated cells. MCF-10F cells treated with P or P+E2 did not show LOH in the any of the markers studied. LOH was strongly associated with the invasion phenotype. Altogether our data indicate that E2 and its metabolites induce in HBEC LOH in loci of chromosomes 13 and 17, that has been reported in primary breast cancer, that the changes are similar to those induced by the chemical carcinogen (BP) and that the genomic changes were not abrogated by antiestrogens.


http://www.sciencedirect.com/science/article/B6T8X-451DKY1-1/2/cbeed4c161a8b8f8ae26359749fb580d9

The association found between breast cancer development and prolonged exposure to estrogen suggests that this hormone is of etiologic importance in the causation of this disease. In order to prove this postulate, we treated the immortalized human breast epithelial cells (HBEC) MCF-10F with 17[beta]-estradiol (E2) for testing whether they express colony formation in agar methocel, or colony efficiency (CE), and loss of ductulogenesis in collagen matrix, phenotypes also induced by the carcinogen benz[a]pyrene (BP). MCF-10F cells were treated with 0.0, 0.007, 70 nM, or 0.25 mM of E2 twice a week for 2 weeks. CE increased from 0 in controls to 6.1, 9.2, and 8.7 with increasing E2 doses. Ductulogenesis was 75 +/- 4.9 in control cells; it decreased to 63.7 +/- 28.8, 41.3 +/- 12.4, and 17.8 +/- 5.0 in E2-treated cells, which also formed solid masses or spherical formations lined by a multilayer epithelium, whose numbers increased from 0 in controls to 18.5 +/- 6.7, 107 +/- 11.8 and 130 +/- 10.0 for each E2 dose. MCF-10F cells were also treated with 3.7 [mu]M of progesterone (P) and the CE was 3.39 +/- 4.05. At difference of E2, P does not impaired the ductulogenic capacity. Genomic analysis revealed that E2-treated cells exhibited loss of heterozygosity in chromosome 11, as detected using the markers D11S29 and D11S912 mapped to 11q23.3 and 11q24.2-25, respectively These results also indicate that E2, like the
chemical carcinogen BP, induces in HBEC phenotypes indicative of neoplastic transformation.


http://www.sciencedirect.com/science/article/B6T8X-3YXC09G-1B/2/a979ca0ab33a3bf8ad6b46dd94e1c076

Four cDNA clones were isolated from a porcine adrenal gland library by using a bovine cytochrome P450(11[beta]) cDNA fragment as a probe. Nucleotide sequences of the four clones overlapped with each other. The deduced amino acid sequences indicated that these clones were derived from a porcine P450(11[beta]) cDNA. Consecutive alignment of these clones covered almost 70% of a coding region of the cDNA, but its 5'-terminus was missing. The adrenal mRNA was reverse-transcribed, and polymerase chain reaction was used to obtain a cDNA fragment including the 5'-terminus. A cDNA constructed from this fragment and the isolated four fragments covered the entire apparent open reading frame of the enzyme, which was thus concluded to comprise 503 amino acids including a putative extension peptide of 24 amino acids at the NH2-terminus. The amino acid sequence was 82% identical to that of bovine P450(11[beta])-3. The cDNA was transfected into COS-7 cells, and steroidogenic activity of the cells was measured. The cells not only converted 11-deoxycorticosterone to corticosterone and 18-hydroxycorticosterone, but also produced aldosterone. Thus we conclude that the primary sequence of porcine P450(11[beta]) which plays a role in the biosynthesis of glucocorticoids as well as mineralocorticoids was determined.


http://www.sciencedirect.com/science/article/B6T8X-3RJX9CF-3/2/51c617ade56865bc15af2ac523438551

Two novel transcripts of human estrogen receptor (ER) have been identified that differ in the 5' untranslated sequence. It has previously been determined that an alternate ER transcript is generated from transcription initiated upstream of the main ER cap site (P1), and utilizes a splice acceptor site at +163. Here we report the isolation of 21 ER clones from a MCF7 cDNA library. Eleven of these clones correspond to transcripts that initiate at the P1 cap site, whereas the remaining 10 clones are derived from two previously unidentified ER transcripts (designated E and H) that both utilize the +163 splice acceptor site. A panel of breast and endometrial carcinoma cell lines were screened by reverse transcriptase-polymerase chain reaction (RT-PCR) for expression of the E and H transcripts. It was found that all ER-positive cell lines expressed both of the novel transcripts. In addition, 10 primary human breast cancers were analyzed, of which six expressed the E transcript and five abundantly expressed the H transcript. These data indicate that expression of ER in human breast cancers can be dependent upon an alternate promoter at least 20 kb upstream of the primary cap site for ER.


http://www.sciencedirect.com/science/article/B6T8X-439WS7B-
Our recent epidemiological study (Ahonen et al., Cancer Causes Control 11(2000) (847-852)) suggests that vitamin D deficiency may increase the risk of initiation and progression of prostate cancer. The nested case-control study was based on a 13-year follow-up of about 19000 middle-aged men free of clinically verified prostate cancer. More than one-half of the serum samples had 25OH-vitamin D (25-VD) levels below 50 nmol/l, suggesting VD deficiency. Prostate cancer risk was highest among the group of younger men (40-51 years) with low serum 25-VD, whereas low serum 25-VD appeared not to increase the risk of prostate cancer in older men (>51 years). This suggests that VD has a protective role against prostate cancer only before the andropause, when serum androgen concentrations are higher. The lowest 25-VD concentrations in the younger men were associated with more aggressive prostate cancer. Furthermore, the high 25-VD levels delayed the appearance of clinically verified prostate cancer by 1.8 years. Since these results suggest that vitamin D has a protective role against prostate cancer, we tried to determine whether full spectrum lighting (FSL) during working hours could increase serum 25-VD concentrations. After 1-month exposure, there was no significant increase in the serum 25-VD level, although there was a bias towards slightly increasing values in the test group as opposed to decreasing values in controls. There was no significant change in the skin urocanic acid production. The possibility to use FSL in cancer prevention is discussed. In order to clarify the mechanism of VD action on cell proliferation and differentiation, we performed studies with the rat and human prostates as well prostate cancer cell lines. It is possible that 25-VD may have a direct role in the host anticancer defence activity, but the metabolism of vitamin D in the prostate may also play an important role in its action. We raised antibodies against human 1[alpha]-hydroxylase and 24-hydroxylase. Our preliminary results suggest that vitamin D is actively metabolised in the prostate. Vitamin D appears to upregulate androgen receptor expression, whereas androgens seem to upregulate vitamin D receptor (VDR). This may at least partially explain the androgen dependence of VD action. VD alone or administered with androgen causes a suppression of epithelial cell proliferation. VD can activate mitogen-activated kinases, erk-1 and erk-2, within minutes and p38 within hours. Also, auto/paracrine regulation might be involved, since keratinocyte growth factor (mRNA and protein) was clearly induced by VD. Based on these studies, a putative model for VD action on cell proliferation and differentiation is presented.


http://www.sciencedirect.com/science/article/B6T8X-43G2YP5-7/2/dceda5a9ab78d6b59645ca83932f8fd7

Although enzymes that catalyze the formation of steroids are well known, less information is available about the enzymes involved in the metabolism of these hormones. Steroid glucuronidation, by UDP-glucuronosyltransferase enzymes, is one mechanism by which steroid hormones can be metabolized and eliminated from a tissue. Previous results suggest that the monkey represents the most appropriate animal model for studying the physiologic relevance of steroid glucuronidating enzymes. The monkey UGT1A01 cDNA clone was isolated by RT-PCR amplification of the liver RNA. The cDNA contains an open reading frame of 1599 bp encoding a protein of 533 residues. The primary structure of monkey UGT1A01 is 95% identical to human UGT1A1. To compare monkey and human UGT1A1 enzymes, both cDNA clones were transfected into HK293 cells and stable cell lines expressing each UGT1A1 protein were established. Western blot analysis of the monUGT1A01-HK293 and hUGT1A1-HK293 cell lines using an anti-UGT1A polyclonal antibody (RC-71) revealed expression of exogenous 55 kDa UGT1 proteins. The transferase activities of monkey and human UGT1A1 proteins were tested with over 60 compounds and were demonstrated to be active on the same compounds. For endogenous compounds only bilirubin and C18 steroids were glucuronidated by these enzymes.
Using microsome preparation (from HK293 cell expressing monkey UGT1A01), the apparent Km values were 13, 5 and 6 [mu]M for the conjugation of estradiol, 2-hydroxyestradiol and 2-hydroxyestrone, respectively, and were very similar to the values obtained with human UGT1A1. Specific RT-PCR analysis demonstrated the expression of monkey and human UGT1A1 transcripts in several tissues including liver, kidney, intestine, prostate, testis and ovary suggesting a contribution of this isoenzyme to estrogen metabolism in the cynomolgus monkey as in human.


Insulin-like growth factor I (IGF-I) is important for gonadal and reproductive functions in mammals, although the physiological role of this growth factor during gonadal development in rodents remains largely unknown. Here, we examined the steady-state levels of IGF-I mRNA by the reverse transcriptase polymerase chain reaction (RT-PCR). IGF-I protein expression was also detected by Western blot. The effect of IGF-I as promoter of 17[alpha]-hydroxylase/C17-20 lyase and 17[beta]-hydroxysteroid dehydrogenase enzyme activity in vitro was evaluated by radioimmunoassay. Onset of IGF-I gene expression was on day E10 (urogenital ridge stage). IGF-I mRNA expression was markedly reduced on days E12 and E13 (testicular differentiation stage). IGF-I transcripts increased on day E14 and their transcription levels were maintained throughout the stages analyzed. Several IGF-I protein bands of 31-100 kDa were observed. Culture experiments demonstrated that 17[alpha]-hydroxyprogesterone and testosterone (T) secretion levels increased in the presence of IGF-I on days E11-E17. Additive effects of IGF-I plus (Bu)2cAMP were also seen during testicular development. It is proposed that IGF-I regulates the expression of key steroidogenic enzymes important for endocrine activity of the testis during prenatal development leading to establishment of the male phenotype and fertility.

The Lancet (36)


Human herpes virus 6 (HHV6) DNA was detected in two of eight fetuses with hydrops and none of ten non-hydropic dead fetuses. Both cases with HHV6 DNA had chromosomal abnormalities. Positive results were confirmed with a second PCR specific for an alternate region of the HHV6 genome. Restriction endonuclease analysis confirmed that the viral DNA was representative of HHV6 type A.

http://www.sciencedirect.com/science/article/B6T1B-451NBBT-D/2/9a3279afd73cc0d16e0edc53550f77db

Summary
Background
Identification of genes and characterisation of their function is an essential step towards understanding complex pathophysiological abnormalities in Down's syndrome. We did a study to investigate abnormalities in gene expression in human neuronal stem cells and progenitor cells from Down's syndrome and control post-mortem human fetal tissue.

Methods
Indexing-based differential display PCR was done on neuronal precursor cells derived from the cortex of a fetus with Down's syndrome, and findings were compared with those of two control samples. Findings were validated against neurosphere preparations from three independent Down's syndrome fetuses and five independent controls by real-time quantitative PCR.

Findings
Results of differential display PCR analysis showed that SCG10—a neuron-specific growth-associated protein regulated by the neuron-restrictive silencer factor REST—was almost undetectable in the Down's syndrome sample. This finding was validated by real-time PCR. We also found that other genes regulated by the REST transcription factor were selectively repressed, whereas non-REST-regulated genes with similar functions were unaffected. Changes in expression of several key developmental genes in the Down's syndrome stem-cell and progenitor-cell pool correlated with striking changes in neuron morphology after differentiation.

Interpretation
Our findings suggest a link between dysregulation of the REST transcription factor and some of the neurological deficits seen in Down's syndrome. Experimental REST downregulation has been shown to trigger apoptosis, which could account for the striking and selective loss of neurons in the differentiated Down's syndrome cell preparations.


http://www.sciencedirect.com/science/article/B6T1B-3Y9H2ND-4Y/2/ff3cc49a315132abaab11e5a750e9df0

Background
Liddle's syndrome is a rare inherited form of hypertension in which mutations of the epithelial sodium channel result in increased renal sodium reabsorption. Essential hypertension in black patients also shows clinical features of sodium retention so we screened black people for the T594M mutation, the most commonly identified sodium-channel mutation.

Methods
In a case-control study, 206 hypertensive (mean age 48.0 [SD 11.8] years, men:women 80:126) and 142 normotensive (48.7 [7.4] years; 61:81) black people who lived in London, UK, were screened for T594M. Part of the last exon of the epithelial sodium-channel [beta] subunit from genomic DNA was amplified by PCR. The T594M variant was detected by single-strand conformational polymorphism analysis of PCR products and confirmed by DNA sequencing.

Findings
17 (8.3%) of 206 hypertensive participants compared with three (2.1%) of 142 normotensive participants possessed the T594M variant (odds ratio [OR]=4.17 [95% CI 1.12-18.25], p=0.029). A high proportion of participants with the T594M variant were women (15 of 17 hypertensive participants and all three normotensive participants), whereas women comprised a lower proportion of the individuals screened (61.2% hypertensive, 57.7% normotensive). However, the association between the T594M variant and hypertension persisted after adjustment for sex and body-mass index (Mantel-Haenszel OR=5.52 [1.40-30.61], p=0.012). Plasma renin activity was significantly lower in 13 hypertensive participants with the T594M variant (median=0.19 ng mL-1 h-1) than in 39 untreated hypertensive individuals without the variant (median=0.45 ng mL-1 h-1 p=0.009).

Interpretation
Among black London people the T594M sodium-channel [beta] subunit mutation occurs more frequently in people with hypertension than those without. The T594M variant may increase sodium-channel activity and could raise blood pressure in affected people.
by increasing renal tubular sodium reabsorption. These findings suggest that the T594M mutation could be the most common secondary cause of essential hypertension in black people identified to date.


http://www.sciencedirect.com/science/article/B6T1B-49K5B3P-32T/2/d1160bfeb2f462146440a72c0a252723

HLA typing contributes to the delays that occur in the search for HLA-matched unrelated donors, and that result in poor patient survival. A new DNA technique for testing DR match between patient and unrelated marrow donors has been assessed. The technique is based on the formation of heteroduplexes between heterologous amplified coding and non-coding DNA sequences during the final annealing stage of the polymerase chain reaction (PCR), and different HLA-DR/Dw types give unique banding patterns (PCR fingerprints) on non-denaturing polyacrylamide gel electrophoresis. HLA-DR matching is by visual comparison of patients' with donors' fingerprints. Identity can be confirmed by mixing donor and recipient DNA before the final stage of the PCR (DNA crossmatching). In an assessment of the technique in 53 unrelated HLA-A and HLA-B matched patient-donor pairs, 42 pairs gave the same results with PCR fingerprinting as with DNA-RFLP analysis. In the 11 other pairs DR/Dw mismatches were detected by PCR fingerprinting but not by the standard DNA-RFLP method; PCR-SSO typing with selected sequence-specific oligonucleotides (SSO) confirmed that mismatches were due to different subtypes of DR4. PCR fingerprinting might thus accelerate the selection of unrelated marrow donors by simplifying the logistics of the donor search.


http://www.sciencedirect.com/science/article/B6T1B-436W05H-H/2/9c30d00629369dcc58f86f59057c5f1c

SummaryBackgroundAbout 1% of white populations are homozygous carriers of an allele of the gene for the CC chemokine receptor 5 (CCR5) with a 32 bp deletion (CCR5[Delta]32), which leads to an inactive receptor. During acute and chronic transplant rejection, ligands for CCR5 are upregulated, and the graft is infiltrated by CCR5-positive mononuclear cells. We therefore investigated the influence of CCR5[Delta]32 on renal-transplant survival.MethodsGenomic DNA from peripheral-blood leucocytes of 1227 renal-transplant recipients was screened by PCR for the presence of CCR5[Delta]32. Demographic and clinical data were extracted from hospital records. Complete follow-up data were available for 576 recipients of first renal transplants. Graft survival was analysed by Fisher's exact test and Kaplan-Meier plots compared with a log-rank test.FindingsPCR identified 21 patients homozygous for CCR5[Delta]32 (frequency 1[middle dot]7%). One patient died with a functioning graft. Only one of the remaining patients lost transplant function during follow-up (median 7[middle dot]2 years) compared with 78 of the 555 patients with a homozygous wild-type or heterozygous CCR5[Delta]32 genotype. Graft survival was significantly longer in the homozygous CCR5[Delta]32 group than in the control group (log-rank p=0[middle dot]033; hazard ratio 0[middle dot]367 [95% CI 0[middle dot]157-0[middle dot]859]).InterpretationPatients homozygous for CCR5[Delta]32 show longer survival of renal transplants than those with other genotypes, suggesting a pathophysiological role for CCR5 in transplant loss. This receptor may be a useful target for the prevention of transplant loss.

http://www.sciencedirect.com/science/article/B6T1B-4FBVXJV-14/2/2f2237832e72339635c19cb0c53ebff7

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause autosomal dominant Parkinson's disease. Few mutations in this gene have been identified. We investigated the frequency of a common heterozygous mutation, 2877510 g->A, which produces a glycine to serine amino acid substitution at codon 2019 (Gly2019 ser), in idiopathic Parkinson's disease. We assessed 482 patients with the disorder, of whom 263 had pathologically confirmed disease, by direct sequencing for mutations in exon 41 of LRRK2. The mutation was present in eight (1.6%) patients. We have shown that a common single Mendelian mutation is implicated in sporadic Parkinson's disease. We suggest that testing for this mutation will be important in the management and genetic counselling of patients with Parkinson's disease.


http://www.sciencedirect.com/science/article/B6T1B-411G2C5-F/2/dc49b8cf18626bde7effa50ad3143051

Background Wilson's disease (WD) is caused by mutations in a P-type ATPase and is associated with copper deposition in liver and brain. The WD protein is present in the trans-Golgi network and may also be imported into mitochondria. The WD protein functions as a P-type copper transporting ATPase in the Golgi but any action in mitochondria is at present unknown. Methods We studied mitochondrial function and aconitase activity in WD liver tissue and compared the results with those in a series of healthy controls and patients without WD. Findings There was evidence of severe mitochondrial dysfunction in the livers of patients with WD. Enzyme activities were decreased as follows: complex I by 62%, complex II-III by 52%, complex IV by 33%, and aconitase by 71%. These defects did not seem to be secondary to penicillamine use, cholestasis, or poor hepatocellular synthetic function. Interpretation The results show that there is a defect of energy metabolism in WD. The pattern of enzyme defects suggests that free-radical formation and oxidative damage, probably mediated via mitochondrial copper accumulation, are important in WD pathogenesis. These results provide a rationale for a study of the use of antioxidants in WD.


http://www.sciencedirect.com/science/article/B6T1B-49K0C6T-3SB/2/d484f26c8dc3e03cbb0c88e3ad058ec9


http://www.sciencedirect.com/science/article/B6T1B-3YTB3WH-B/2/ff8ab7a1c64ccd0f063daa07e51e5b62
Background The concentration of T-cell receptor-rearrangement excision DNA circles (TREC) in peripheral-blood T cells is a marker of recent thymic emigrant αβ T cells. We studied the predictive ability of measurements of TREC for clinical outcome in HIV-1-infected individuals.

Methods We measured TREC in peripheral-blood mononuclear cells with a real-time PCR assay. We studied 131 Greek participants in the Multicenter Hemophilia Cohort Study who had known HIV-1 seroconversion dates. The prognostic value of baseline TREC, CD4 T-cell count, and HIV-1 RNA concentration was assessed by Kaplan-Meier and Cox's regression analysis.

Findings Four participants had progressed to AIDS by first blood sampling. Among the remaining 127 individuals, the median value of TREC per 10^6 cells was 6900 (IQR 2370-15 604). Baseline TREC values were lower in the 53 who progressed to AIDS than in those who did not (geometric mean 2843 [95% CI 1468-5504] vs 6560 [4723-9113] TREC per 10^6 cells; p=0.017).

The relative hazard of AIDS, adjusted for plasma viral load, CD4 T-cell count, and age at seroconversion was 1.44 (95% CI 1.04-2.01; p=0.031) per ten-fold increase in TREC; that for death was 1.52 (1.12-2.06; p=0.007). The adjusted relative hazards of death were 2.91 (1.91-4.44; p-interaction) 1.81 (1.12-2.91; p=0.01) per ten-fold increase in TREC. The concentration of TREC in the peripheral T-cell pool complements HIV-1 RNA load and CD4 T-cell count in predicting the rate of HIV-1 disease progression. Recent thymic emigrants have a role in the pathogenesis of HIV-1 disease.

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http://www.sciencedirect.com/science/article/B6T1B-3XYFJJ5-F/2/2a665997669f596831e59e09a3773e9

Background Sulpha drugs are widely used for the treatment and long-term prophylaxis of Pneumocystis carinii pneumonia (PCP) in HIV-1-infected individuals. Sulpha resistance in many microorganisms is caused by point mutations in dihydropteroate synthase (DHPS), an enzyme that is essential for folate biosynthesis. We assessed whether mutations in the DHPS gene of P carinii were associated with exposure to sulpha drugs and influenced outcome from PCP.

Methods We studied bronchoalveolar samples collected in 1989-99 from a prospective cohort of HIV-1-infected patients who had PCP. In 144 patients with 152 episodes of PCP, we analysed portions of DHPS using PCR and direct sequencing. The relation between survival, P carinii DHPS mutations, and other predictors of treatment failure was assessed by Kaplan-Meier and multivariate Cox regression analysis.

Findings P carinii DHPS mutations were found in 31 (20.4%) of 152 PCP episodes. 3-month survival was significantly lower in patients infected with mutant P carinii DHPS strains than in those with wild-type strains (p=0.002). After adjustment for other prognostic variables, presence of DHPS mutations remained the most important predictor of mortality (hazard ratio 3.1 [95% CI 1.2-8.1]). DHPS mutations were significantly more common in patients who had previous exposure to sulpha drugs (18 of 29 [62%]) than in those who had no exposure (13 of 123 [10.5%]; pDHPHPS mutations (p=0.01 for trend) was closely correlated with the rate of previous or current use of sulpha drugs as chemoprophylaxis. Interpretation Mutations in DHPS are associated with impaired prognosis in PCP, and may develop as a result of exposure to sulpha drugs.


http://www.sciencedirect.com/science/article/B6T1B-49K2BM-1H1/2/53214cf820778f8cbb3f5b51735ef164

We describe a rapid method of HLA class I typing using the polymerase chain reaction and
oligonucleotide hybridisation that eliminates the requirements for viable lymphocytes and allows subtypes to be defined. We have used this to demonstrate that the predominant subtype of HLA-B27 in the Gambia, West Africa, is HLA-B*2703, which is very rare or absent in other racial groups. This subtype differs from the common Caucasian HLA-B27 subtypes in its recognition by cytotoxic T cells. We propose that HLA-B*2703, unlike other HLA-B27 subtypes, may not be associated with ankylosing spondylitis, thus accounting in part for the rarity of this condition in black populations.


http://www.sciencedirect.com/science/article/B6T1B-49HFW1P-4CR/2/35da56af530a8c756d89df6d86d4690

Mouse preimplantation embryos were accurately diagnosed as normal or mutant at the beta-major haemoglobin locus by amplification of specific DNA sequences in a single cell. A DNA sequence containing the whole of exon 3 and some 3’ untranslated sequences within the beta-major haemoglobin gene was amplified in single blastomeres by means of the polymerase chain reaction (PCR). Blastomeres were removed from embryos of four to eight cells from normal BALB/c mice and from mutant (thalassaemic) BALB/c mice homozygous for a deletion of the whole beta-major haemoglobin gene. The sensitivity of the amplification procedure was enhanced by the sequential use of two sets of oligonucleotide primers for 30 cycles of amplification each, the second pair being located within the segment amplified by the first pair. The product (204 base-pairs) could be easily visualised in ethidium bromide-stained agarose gels. Stringent precautions to prevent contamination were taken, and with these precautions the PCR amplification procedure could be carried out under normal laboratory conditions. These procedures for diagnosis of genetic disease before


http://www.sciencedirect.com/science/article/B6T1B-48KVF5R-B/2/3d77a37ffeeae4d8f7f353993dfe2401

SummaryBackgroundHyperhomocysteinaemia occurs in several genetically determined and acquired disorders, and is highly prevalent in patients with uraemia. In these disorders, homocysteine precursor S-adenosylhomocysteine, a powerful competitive inhibitor of S-adenosylmethionine-dependent methyltransferases, is increased, suggesting unbalanced methylation. We aimed to investigate whether DNA hypomethylation is present in patients with uraemia who also have hyperhomocysteinaemia and whether regulation of specific classes of genes, dependent on DNA methylation, is compromised.MethodsWe selected men with hyperhomocysteinaemia and uraemia who were having standard haemodialysis treatment, and compared them with healthy male controls. We measured the homocysteine concentration from plasma samples and obtained DNA and RNA samples from peripheral mononuclear cells. DNA methylation was assessed by cytosine extension assay and by Southern blotting. Allelic expression of pseudoautosomal and imprinted genes was investigated by analysis of suitable restriction fragment length polymorphisms.FindingsTotal DNA hypomethylation was higher in patients than in controls (z score -4[middle dot]593, p=0[middle dot]0006) and allelic expression was changed in both sex-linked and imprinted genes. The shift from monoallelic to biallelic expression was dependent on homocysteine concentrations. Folate therapy, a common method
to reduce hyperhomocysteinaemia, restored DNA methylation to normal levels and corrected the patterns of gene expression.

Interpretation
Our results suggest that hyperhomocysteinaemia affects epigenetic control of gene expression, which can be reverted by folate treatment. Our data support the hypothesis that the toxic action of homocysteine can be mediated by macromolecule hypomethylation.


http://www.sciencedirect.com/science/article/B6T1B-49M0JHS-20R/2/a055fc745e2c578f5f4c912ed171d1b9


http://www.sciencedirect.com/science/article/B6T1B-46HNW1W-D/2/3593cd67943d813e92da0a08f435bedd

Summary
Background
The contact system triggers the kallikrein-kinin cascade, liberating bradykinin from high-molecular-weight kininogen. Effectors of the contact system have proinflammatory and vasoactive properties. Vasculitis is a condition characterised by inflammation around vessel walls, leading to secondary tissue damage for which the underlying molecular mechanisms are poorly understood. Our aim was to investigate contact-system activation in children with vasculitis.

Methods
We compared 17 children, aged 4-19 years, with vasculitis, engaging the skin, joints, intestines, or kidneys, with 21 controls, aged 2-18 years. We analysed proteolysis of high-molecular-weight kininogen by immunoblotting. Plasma bradykinin concentrations were quantified by ELISA. Kidney and skin biopsies were stained in situ for kinins. Concentrations of heparin binding protein (HBP) were quantified by ELISA.

Findings
We noted extensive proteolysis of high-molecular-weight kininogen in the plasma of 13 of 17 patients, but in only one of 21 controls (pInterpretation
Activation of the contact system could play a part in the pathogenesis of vasculitis, and explain the inflammation, pain, vasodilatation, and oedema seen in patients.


http://www.sciencedirect.com/science/article/B6T1B-4BC1S83-J/2/12d29dfac4096e6364ae164ccf25c3a3

Summary
Testicular germ-cell tumours (TGCTs) are the most common malignant diseases among young men, with peak incidence at age 20-40 years. We developed a DNA tumour marker for TGCTs based on the unmethylated DNA profile of a neoplasm. The 5' end of the XIST gene is mainly hypomethylated in TGCTs irrespective of XIST expression. Male somatic cells, however, show complete methylation through the CpG sites, including the minimum promoter and XIST-conserved repeats. Identification of a XIST unmethylated fragment in male plasma might be diagnostic for TGCTs.

http://www.sciencedirect.com/science/article/B6T1B-3W9KVPB-C/2/3cc53a874d0f2ca0139bb43f8b86ef5

Background A five-fold increase in risk of stent thrombosis in carriers of A1/A2 (Leu33Pro) polymorphism of glycoprotein IIIa has been described. Whether this increased procedural risk applies to other coronary interventions is unknown. We investigated the role of A1/A2 polymorphism as a putative risk factor.

Methods We genotyped 1000 consecutive patients with angiographically confirmed coronary-artery disease and 1000 controls matched for age and sex. 653 of the 1000 patients received interventions (271 coronary angioplasty, 102 directional coronary atherectomy, and 280 stenting) and were assessed for a 30-day composite endpoint of need for target-vessel revascularisation, myocardial infarction, and death.

Findings The composite endpoint occurred in 41 (6.3%) patients. There was no evidence that the A2 allele was associated with excess procedural risk (relative risk 1.36 [95% CI 0.70-2.70], p=0.37). Nor, in subgroup analyses, did A2 predict events that complicated coronary angioplasty (1.17 [0.40-2.70]), directional coronary atherectomy (1.50 [0.30-8.70]), or stenting (1.45 [0.60-3.50]). Neither heterozygotes (A1/A2) nor homozygotes (A2/A2) were over-represented in any subgroup, including those with acute coronary syndromes, early disease manifestation (age

Interpretation A1/A2 polymorphism is not a major risk factor for 30-day adverse events that complicate coronary angioplasty, directional coronary atherectomy, or stenting. Furthermore, A1/A2 polymorphism has no apparent impact on more chronic processes such as atherogenesis of the coronary arteries.


http://www.sciencedirect.com/science/article/B6T1B-49K57JH-18X/2/42d0cf8fde351239acb240791367319

Some types of reused dental equipment, especially handpieces and their attachments for drilling and cleaning teeth, might be responsible for cross-contamination if patient material were to lodge temporarily in difficult-to-disinfect internal mechanisms. This possibility is worrisome with respect to transmission of hepatitis B and human immunodeficiency viruses (HBV, HIV). Previous cross-contamination studies have relied on laboratory experiments with bacteria or dye tracers. To assess possible risks more thoroughly, we tested 30 new prophylaxis angles and 12 new high-speed handpieces to see whether they would take up and expel contaminants in laboratory and clinical trials.

In treatments of three patients, including two infected with HIV, human-specific DNA ([beta]-globin, HLA DQ[alpha]) and HIV proviral DNA were detected inside or coming back from the devices. Similarly, when handpieces were operated in contact with blood pooled from HBV-infected patients, HBV DNA was detected in samples taken from inside the equipment and from their attached air/water hoses. When we used bacteriophage [phi]X174 as a model virus in laboratory tests, many infective viral particles were recovered from internal mechanisms of handpieces, their connecting air/water hoses, and from water spray expelled when the equipment was reused. We recommend that reused high-speed, air-driven handpieces and prophylaxis angles should be cleaned and heat-treated between patients. Further studies are needed to determine ways of eliminating the risks associated with exhaust hoses and air/water input lines.

Summary

Background

Concerns have been raised about emergence of ganciclovir resistance as a result of the advent of both routine oral ganciclovir prophylaxis and highly potent immunosuppression. We retrospectively assessed the occurrence of ganciclovir-resistant cytomegalovirus disease among transplant recipients who had received oral ganciclovir prophylaxis and highly potent immunosuppression.

Methods

We studied 240 recipients of liver, kidney, or pancreas transplants. Antiviral susceptibility testing of blood cytomegaloviral isolates was done when patients failed to respond to intravenous ganciclovir treatment for symptomatic cytomegalovirus infection. Portions of the UL97 gene associated with ganciclovir resistance were sequenced in cytomegalovirus isolates with phenotypic resistance to ganciclovir.

Findings

Ganciclovir-resistant cytomegalovirus disease developed in five (7%) of 67 seronegative recipients of cytomegalovirus-seropositive organs (D+/R-) compared with none of 173 seropositive recipients (p=0.002). Among the 25 (10.4%) patients who developed cytomegalovirus disease within 1 year after transplantation, five had ganciclovir-resistant cytomegalovirus disease. Among D+/R- transplant recipients, ganciclovir-resistant cytomegalovirus disease was more common among the group receiving the most potent immunosuppression—ie, recipients of kidney and pancreas or pancreas alone (four of 19) compared with all other transplant recipients (one of 48, p=0.02). Ganciclovir-resistant cytomegalovirus disease was diagnosed at a median of 10 months after transplantation (range 7-12) after lengthened exposure to ganciclovir, was associated with previously described mutations of the UL97 gene, and led to serious clinical complications.

Interpretation

Ganciclovir-resistant cytomegalovirus is an important cause of late morbidity among D+/R- patients, especially among D+/R- patients, are warranted.


Detection of Pneumocystis carinii by the polymerase chain reaction (PCR) may facilitate noninvasive diagnosis of P carinii pneumonia and study of its epidemiology. We have compared the sensitivity and specificity of two PCR methods with those of conventional staining for detection of P carinii in induced sputum, bronchoalveolar lavage fluid (BAL), and blood. Of 71 sputum samples, 17 were from patients with microbiologically confirmed P carinii pneumonia. A nested PCR method correctly identified the presence of P carinii in all 17 (100% sensitive, 95% confidence interval [CI] 81-100%) and found no organisms in 50 of 54 microbiologically negative samples (93% specific, 95% CI 82-98%). PCR with a single primer pair was 71% sensitive (44-90%) and 94% specific (85-99%). The sensitivity of conventional staining methods (direct and indirect fluorescence antibody and toluidine-blue-O tests) was significantly less (38-53%) than that of nested PCR (pP carinii was detected in BAL or sputum from 10 immunocompromised patients without microbiological evidence of P carinii pneumonia, which suggests that symptom-free carriers or subclinical infection can exist. P carinii was detected by nested PCR in blood from 2 of 3 patients with disseminated pneumocystosis but in only 1 of 11 patients with P carinii infection restricted to the lungs. Nested PCR on induced sputum is more sensitive than conventional staining methods for the diagnosis of P carinii pneumonia and provides a non-invasive method of detecting disseminated disease.

http://www.sciencedirect.com/science/article/B6T1B-4D8V8SH-19/2/97c2d16510ef0a7f6f0d4e0da9896ab5

Summary

Background

Mutations in the gene encoding mitochondrial DNA polymerase [gamma] (POLG), the enzyme that synthesises mitochondrial DNA (mtDNA), have been associated with a mitochondrial disease—autosomal dominant or recessive progressive external ophthalmoplegia—and multiple deletions of mtDNA. Mitochondrial dysfunction is also suspected to participate in the pathogenesis of Parkinson's disease. However, no primary gene defects affecting mitochondrial proteins causing mendelian transmission of parkinsonism have been characterised. We aimed to analyse the gene sequence of POLG in patients with progressive external ophthalmoplegia and their healthy relatives.

Methods

In seven families of various ethnic origins we assessed patients with progressive external ophthalmoplegia and unaffected individuals by clinical, biochemical, morphological, and molecular genetic characterisation and positron emission tomography (PET).

Findings

We recorded mutations in POLG in members of all seven families. Clinical assessment showed significant cosegregation of parkinsonism with POLG mutations (pPOLG gene defect resulted in secondary accumulation of mtDNA deletions in patients' tissues).

Interpretation

Dysfunction of mitochondrial POLG causes a severe progressive multisystem disorder including parkinsonism and premature menopause, which are not typical of mitochondrial disease. Cosegregation of parkinsonism and POLG mutations in our families suggests that when defective, this gene can underlie mendelian transmission of parkinsonism.

Relevance to practice

Awareness that mitochondrial POLG mutations can underlie parkinsonism is important for clinicians working in diagnosis of movement disorders, as well as for studies of the genetics of Parkinson's disease. Further, progressive external ophthalmoplegia with muscle weakness and neuropathy can mask symptoms of parkinsonism, and clinicians should pay special attention to detect and treat parkinsonism in those individuals.


http://www.sciencedirect.com/science/article/B6T1B-41PVXTPM/2/287ce1e5dd5a24f5f33b7a7feef5dcd8b

Summary

The CCR5-[Delta]32 deletion polymorphism (CCR5-[Delta]32) was investigated for linkage and association to asthma and atopy using two panels of nuclear families containing 1284 individuals. No statistically significant linkage to asthma or wheeze or atopy was observed in either of the two panels of families. Multiallelic transmission disequilibrium tests (TDT) of the combined data found no significant association for atopy (52 independent alleles transmitted, 51 non-transmitted) or asthma/wheeze (39 transmitted, 44 non-transmitted). Although functional evidence might suggest that CCR5 is a good candidate gene for atopic asthma, this study provides no genetic evidence from CCR5-[Delta]32 polymorphism to support this hypothesis.


http://www.sciencedirect.com/science/article/B6T1B-49M0N8R-373/2/1e7485d6a509b2e1d656648127268f79

A 680 base-pair sequence of the human [beta]-haemoglobin gene was reproducibly amplified in
individual unfertilised human oocytes and in first polar bodies isolated from them. Specificity and 
sensitivity of amplification were achieved by two sequential reactions with two sets of primers, 
amplifying first a 725 base-pair sequence and secondly a 680 base-pair sequence from within the 
first amplified fragment. A restriction enzyme digest of the DNA amplified from a single oocyte 
with the endonuclease Dde I confirmed the identity of the amplified [beta]-haemoglobin fragment; 
this technique provides a diagnostic test for the genetic defect responsible for sickle cell anaemia. 
Analysis of the DNA from the first polar body may enable detection of such defects in unfertilised 
eggs from carrier women. Selection of eggs without the defect for fertilisation may therefore 
obliterate the need for diagnostic procedures on embryos.

347(9007): 1014.

http://www.sciencedirect.com/science/article/B6T1B-4B8JK7N-169/2/02abac5f95591fd0c1d66dda15a92f07

SummaryBackground Batten's disease is the most common progressive encephalopathy of 
childhood in Western countries. The major mutation is a 1 kb deletion, which is carried by 81% of 
Batten's disease patients. We report on the use of direct gene analysis in the prenatal diagnosis 
of this disease.Methods and findings A Finnish woman with a son with Batten's disease came for 
genetic counselling for her current pregnancy. Electron microscopy of a chorionic villus sample 
gave suggestive findings. We used PCR to look for the intragenic microsatellite marker D16S298; 
96% of Finnish Batten's disease patients carry allele 6 at this marker. The fetus and the affected 
son both carried the same high-risk genotype, 6/6. Both were homozygous for the 1 kb deletion. 
The pregnancy was terminated. Electron microscopy of the fetus showed typical Batten's disease 
changes. Interpretation We have successfully used direct gene analysis in the prenatal diagnosis 
of Batten's disease.

by rapid screening of whole essential region of factor VIII gene." The Lancet 337(8742): 635.

http://www.sciencedirect.com/science/article/B6T1B-49K2KBM-1GY/2/54e17c75b8ae4185bf0bbc088fa13a1

In an attempt to replace the existing, DNA-based, 50% effective, carrier and prenatal diagnoses 
of haemophilia A with the 100% successful direct detection of defective genes, a new procedure 
was developed to screen and identify mutations in all the essential regions of the factor VIII gene 
(putative promoter, coding sequence, and the cleavage and polyadenylation region). Genomic 
DNA and cDNA obtained by reverse transcription of the leaky mRNA found in peripheral 
lymphocytes were amplified by means of the polymerase chain reaction to yield a set of eight 
segments comprising the essential gene sequences. The segments were then screened 
individually for mutations by the amplification mismatch detection method, which detects and 
locates any type of sequence discrepancy between the test DNA and the control probe by 
cleavage of the probe at the site of mismatches. Two haemophilia A patients were studied. The 
first showed two single-base changes: one (substitution of tryptophan 2229 by cysteine in the C2 
domain) is the probable cause of the disease, since it affects a conserved residue of factor VIIIa, 
whereas the other (the conservative substitution of aspartic acid at position 1241 by glutamic 
acid) occurs in a domain (B) irrelevant to factor VIII activity. The second patient showed a 
complete failure of pre-mRNA splicing due to a single-base substitution that changes the 
obligatory AG acceptor splice site of intron 5 to GG. The method characterises the gene defect in 
10 days or less and should lead to the rapid accumulation of information on the molecular biology 
of haemophilia A.

http://www.sciencedirect.com/science/article/B6T1B-49M0MHX-2WH/2/ee54c2e8ae7ce7266c4d57e02db9e134

The application of the amplification refractory mutation system (ARMS) to the detection of individual [beta]-thalassaemia mutations in heterozygous parents and at risk fetuses has been assessed in Indian and Cypriot immigrant populations in the UK. 100 first trimester prenatal diagnoses have been done, entailing the detection of 17 different mutations. The method, which allows the determination of the mutations in both parental and fetal DNA on the same day, should have wide application to the carrier detection and prenatal diagnosis of monogenic diseases with heterogeneous molecular defects.


http://www.sciencedirect.com/science/article/B6T1B-49KJXD5-147/2/a76c783b8db34652886a5a683022622e


http://www.sciencedirect.com/science/article/B6T1B-3TXSRB9-4/2/7ca76ab386c17ac1ef25bff5bb5f0db

Background Despite multiple exposures to HIV-1, some individuals remain uninfected, and their peripheral-blood mononuclear cells (PBMC) are resistant to in-vitro infection by primary HIV-1 isolates. Such resistance has been associated with a homozygous 32-base-pair deletion ([Delta]32) in the C-C chemokine receptor gene CCR5. We examined other mutations of the CCR5 gene that could be associated with resistance to HIV-1 infection.Methods We assessed the susceptibility of PBMC to in-vitro infection by HIV-1 isolates that use the CCR5 as the major coreceptor for viral entry in 18 men who had frequent unprotected sexual intercourse with a seropositive partner. We also did genotypic analysis of CCR5 alleles. One of the 18 exposed but uninfected men (who we refer to as ExU2) showed total resistance to in-vitro infection by CCR5-dependent viruses, and was found to carry a CCR5 [Delta]32 allele and a single point mutation (T->A) at position 303 on the other allele. To find out whether the CCR5 mutation was restricted to ExU2's family or existed in the general population, we did genetic analyses of the CCR5 genotype in ExU2's father and sister and also in 209 healthy blood donors who were not exposed to HIV-1.Findings The m303 mutation found in ExU2 introduced a premature stop codon and prevented the expression of a functional coreceptor. The family studies revealed that the m303 mutant allele was inherited as a single mendelian trait. Genotype analysis showed that three of the 209 healthy blood donors were heterozygous for the mutant allele.Interpretation We characterise a new CCR5 gene mutation, present in the general population, that prevents expression of functional coreceptors from the abnormal allele and confers resistance to HIV-1 infection when associated to the [Delta]32 CCR5 mutant gene.
Rapid detection of deletion and duplication mutations that cause Duchenne and Becker muscular dystrophy was achieved in patients and carriers after amplification of small amounts of mRNA from peripheral blood lymphocytes. The entire coding region of the dystrophin mRNA was amplified in 10 sections by reverse transcription and nested polymerase chain reaction, and the products were directly visualised on acrylamide minigels with ethidium staining. Major structural gene mutations were identified by the appearance of a band of different size to that of the wild type. The altered band was readily detected in all patients and heterozygous relatives. This nonradioactive test of venous blood samples can be used for unambiguous and rapid identification of virtually all carriers of deletions or insertions within the dystrophin gene.

Background Despite sensitive antibody-based blood-donor screening, a residual risk of transfusion-transmitted viral infections exists. Only direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures. We investigated the feasibility and efficacy of routine screening of donors for hepatitis C virus (HCV), hepatitis B virus (HBV), and HIV-1 by PCR.

Methods For PCR testing, individual donor plasma samples were pooled (96 x 100 [mu]L) overnight by two automatic pipetting machines. Viruses were concentrated by centrifugation and nucleic acids were extracted. HCV PCR was done on the Cobas Amplicor system (Hoffmann-La Roche, Mannheim, Germany). HBV and HIV-1 sequences were amplified by single (non-nested) in-house PCRs and detected by agarose-gel electrophoresis. Detection limits were 1000-5000 genome equivalents/mL in the donor blood.

Findings PCR testing was done in parallel to antibody screening with a maximum throughput of 3000 samples in 7-8 h. Positive samples were identified 1-2 days later. 111 of 373423 donations (107 of 4500 pools) were PCR and antibody/antigen-confirmed positive. We found one HCV PCR-positive antibody-negative donation with normal alanine aminotransferase and one HCV PCR-positive donation with an elevated alanine aminotransferase (100 IU), which was negative in the AxSYM 2.0 and Matrix 1.0, but positive after control in the Abbott Prism test (Abbott GmbH, Wiesbaden, Germany). Interpretation PCR is a suitable and fast blood-donor screening procedure and contributes to a reduction in viral transmission by transfusion of blood components. In our selected donor population, the yield of detected contaminated donations from donors in the time window in which they are highly infectious but do not have any symptoms or detectable antigen and antibody concentrations (diagnostic window), confirms theoretical estimates.

Oligonucleotide primers and probes were used in the polymerase chain reaction to amplify Pneumocystis carinii specific DNA sequences from alveolar lavage samples from 47 diagnostic bronchoscopies. No P carinii DNA was found in lavage from 10 immunocompetent patients; only low levels were found in 3 of 13 samples from immunosuppressed individuals without P carinii pneumonia (PCP), and the highest levels, readily demonstrated by simple ethidium bromide staining, were found in all of 16 samples from immunosuppressed patients with PCP confirmed by means of standard silver staining and in 4 from patients with clinical PCP but negative silver staining. DNA amplification provides a highly sensitive and specific technique for the identification of Pcarinii that should be valuable in epidemiological studies on this parasitic infection and in diagnosis.


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http://www.sciencedirect.com/science/article/B6T1B-3WK3BSG-B/2/f186398e4702485929c6f6857d84871f

Background Mutations in the genes on chromosome 16p12 that encode the [beta] and [gamma] subunits of the epithelial sodium channel (SCNNIB and SCNNIG, respectively) have been linked with rare sodium-dependent forms of low and high blood pressure. Other DNA variants in or around these genes may contribute to variation in blood pressure and the risk of coronary heart disease and stroke.

Methods We studied 286 white families from the general population in Victoria, Australia. Each family comprised both parents and two natural children. All participants were genotyped at chromosome 16p12 by use of four highly polymorphic microsatellite markers. Quantitative phenotype measurements were correlated with genotype in identity-by-descent sibling-pair linkage analyses.

Findings We found significant linkage between systolic blood pressure and chromosome 16p12 after parametric analyses (p=0.0003) and non-parametric analyses (p=0.001). The mean difference in systolic blood pressure between siblings identical-by-descent at these loci was half as large (7.1 mm Hg) as the difference between siblings non-identical at these loci (14.0 mm Hg, p=0.001). No linkage between chromosome 16p12 and diastolic blood pressure or body-mass index was observed.

Interpretation Chromosome 16p12 and the SCNNIB and SCNNIG genes are implicated in the physiological variation of systolic blood pressure. Our findings are important in explaining individual cardiovascular risk within the general population.


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While there is evidence that hepatitis C virus (HCV) does not cause fulminant non-A, non-B hepatitis, the causal agent remains unknown. To evaluate the role of hepatitis B virus (HBV) in
this disease, we used a two-step polymerase chain reaction (PCR) to amplify the surface and core regions of HBV DNA in serum and liver samples taken prospectively from twenty-six patients (mean age 36 years, range 1 to 64) with acute hepatic failure undergoing liver transplantation. HBV DNA was absent from the serum of all patients before transplantation. Seventeen patients were diagnosed as having non-A, non-B hepatitis because they lacked serological evidence of hepatitis A virus or HBV infection. Liver samples were taken from twelve of these patients, and six samples were positive for HBV DNA. By contrast HBV DNA was not detected in liver from three patients with acute liver failure caused by hepatitis A or toxins. HCV RNA was not found in pretransplant samples by PCR. Four of the six patients with detectable HBV DNA in liver and presumptive non-A, non-B hepatitis had detectable HBV DNA in serum after transplantation. One additional patient who did not donate pretransplant liver had HBV DNA in a post-transplant serum sample. Thus, HBV DNA was present before or after transplantation in seven of seventeen patients with apparent non-A, non-B hepatitis. Three of five patients with detectable post-transplant serum HBV DNA were serologically positive for HBV surface antigen. These findings indicate that HBV may be a common cause of fulminant hepatic failure in patients lacking serological evidence of HBV infection.


http://www.sciencedirect.com/science/article/B6T1B-49V58KN-B/2/a1dfc425a7e308d963895e91bba51c7f

SummaryBackground Del22q11.2 syndrome is the most frequent known chromosomal microdeletion syndrome, with an incidence of 1 in 4000-5000 livebirths. It is characterised by a 3-Mb deletion on chromosome 22q11.2, cardiac abnormalities, T-cell deficits, cleft palate facial anomalies, and hypocalcaemia. At least 30 genes have been mapped to the deleted region. However, the association of these genes with the cause of this syndrome is not clearly understood. Methods To test for the chromosomal deletion at 22q11.2, we did fluorescence in-situ hybridisation analysis with ten probes on 22q11.2 in 235 unrelated patients with clinically diagnosed del22q11.2 syndrome. To investigate mutations in the coding sequence of TBX1, we also did genetic analysis in 13 patients from ten families who have the 22q11.2 syndrome phenotype but no detectable deletion of 22q11.2. Findings 96% (225 of 235) of patients had a defined 1[middle dot]5-3-Mb deletion at 22q11.2. We identified three mutations of TBX1 in two unrelated patients without the 22q11.2 deletion—one with sporadic conotruncal anomaly face syndrome/velocardiofacial syndrome and one with sporadic DiGeorge's syndrome—and in three patients from a family with conotruncal anomaly face syndrome/velocardiofacial syndrome. We did not record these three mutations in 555 healthy controls (1110 chromosomes; p

InterpretationOur results suggest that the TBX1 mutation is responsible for five major phenotypes in del22q112 syndrome. Therefore, we conclude that TBX1 is a major genetic determinant of the del22q11.2 syndrome.


http://www.sciencedirect.com/science/article/B6T1B-3XFTGMD-D/2/8bd731cae2532a0c2241dab7b74cf73e

Background Resistance of HIV-1 to antiretroviral drugs is the main cause of antiretroviral-treatment failure. We assessed the transmission of drug-resistant variants among individuals with primary HIV-1 infection. Methods Population-based sequencing of the viral reverse-transcriptase and protease genes derived from plasma viral RNA was done in 82 consecutive individuals with
documented primary HIV-1 infection from January, 1996, to July, 1998. Phenotypic resistance to protease inhibitors was assessed by recombinant virus assay in individuals with two or more mutations associated with resistance to protease inhibitors. Findings Zidovudine-resistance mutations were detected in seven (9%) of 82 individuals. Mutations associated with resistance to other reverse-transcriptase inhibitors (RTIs) were detected in two individuals. Primary-resistance mutations associated with protease inhibitors (V82A, L90M) were detected in three (4%) of 70 individuals; two of these had also RTI-resistance mutations. Decreased sensitivity to three or four protease inhibitors was seen in three individuals, one of whom was infected with HIV-1 variants that harboured 12 mutations associated with resistance to multiple RTI and protease inhibitors. Interpretation To introduce the best antiretroviral treatment, resistance testing should be done in recently HIV-1-infected individuals.

The Science of The Total Environment (2)


http://www.sciencedirect.com/science/article/B6V78-44X036P-3/2/f9f16bfa7eec7af633dd8eb1f70771ca

Bacteria of the genus Aeromonas are ubiquitous in aquatic environments, including mineral drinking and thermal waters. Motile species are related to different diseases, mostly gastrointestinal disorders. Criteria for Aeromonas pathogenicity in humans and animals are still unclear and neither is the relationship between production virulence and pathogenicity factors. In the present study, strains of Aeromonas hydrophila, from 61 samples of bottled mineral waters and 23 thermal Italian sources have been isolated and identified by biochemical tests, for toxicity and detection of the aerolysin gene by the Polymerase Chain Reaction (PCR). Six strains were isolated from the mineral waters and were found to be cytotoxic and in possession of the aerolysin gene. For the twelve strains isolated from thermal waters, seven were cytotoxic and eleven contained the aerolysin gene.


http://www.sciencedirect.com/science/article/B6V78-3YVM1DK-F/2/42fb9b90f24f6ff003ec8adb32b72ebb

A new methodology has been developed to assess cytochrome P4501A expression in two South Atlantic Spanish fish, guilthead seabream (Sparus aurata) and grey mullet (Liza aurata), used as pollution bioindicators. Degenerate oligos were used to amplify by reverse transcription and PCR (RT-PCR) specific cyp1A cDNA sequences, used subsequently to design specific primers to get the full cDNA by rapid amplification of cDNA ends. A new assay has been developed to quantitate cyp1A expression by RT-PCR in an automated DNA sequencer. The effect of [beta]-naphthoflavone inducing biotransformation has been used to compare three distinct pollution biomarkers: EROD activity, ELISA determination of CYP1A, and 2-aminoanthracene (2-AA) activation. Immunodetection by ELISA or Western blot was inconsistent in S. aurata and L. aurata. EROD activity yielded satisfactory results; the higher induction was observed by
bioactivation of 2-AA to mutagens detected with strain BA149 of Salmonella typhimurium, in agreement with the high sensitivity previously described for this biomarker. The present paper summarizes the current status of our research.

The Veterinary Journal  


http://www.sciencedirect.com/science/article/B6WXN-4DJBRB8-1/2/e2757eb0a9cada2c9e3fdd62676907d0

A study was carried out in the South of Italy to assess the role of clostridia in neonatal diseases of lambs and kids. Eighty-seven lambs and 15 kids belonging to 25 flocks were examined and Clostridium perfringens was the microorganism most commonly identified. C. perfringens isolates were analysed by polymerase chain reaction (PCR), in order to determine the prevalence of the genes cpa, cpb, cpb2, etx, iap and cpe. The most prevalent toxin-type of C. perfringens was found to be type A found in 84% of the cases with clostridial enterotoxaemia. No C. perfringens type B, C or E were found. C. perfringens type D was isolated in 16% of the cases. About 24% of the isolates were cpb2 positive. The prevalence of cpb2 across the different C. perfringens types varied. The [beta]2-toxin gene cpb2 was detected in 4/21 (19%) type A isolates, in 1/2 type D isolates, and in 1/2 type DE (cpe-carrying type D) isolates. The high rate of positivity to cpb2 among the isolates suggests that a vaccine based on the [beta]2-toxin, should be included in the vaccination schedule of the animals to confer adequate protection and to prevent the disease.

Grom, J., P. Hostnik, et al. "Molecular detection of BHV-1 in artificially inoculated semen and in the semen of a latently infected bull treated with dexamethasone." The Veterinary Journal In Press, Corrected Proof  

http://www.sciencedirect.com/science/article/B6WXN-4F6SSH-1/2/2c259616d4733bcc5480fbe7da5b0060

Two polymerase chain reaction (PCR) assays specific for glycoprotein B (gB) and glycoprotein E (gE) gene detection, respectively, were adopted for the detection of bovine herpesvirus-1 (BHV-1) in naturally infected bulls. The methods were tested on bovine semen artificially inoculated with BHV-1 and were compared with an optimised virus isolation method. Raw and extended semen samples were diluted in minimal essential medium (MEM) and spiked with equal dose of BHV-1. The extended semen was found to be more toxic for the cells than the raw semen, while the viral DNA could be detected by the PCR method in all tested dilutions of raw and extended semen samples. The sensitivity of both methods was compared also for BHV-1 detection in semen, nasal swabs and leucocytes of a seropositive bull in a different time period after virus reactivation with dexamethasone treatment. The sensitivity of virus detection by the PCR method was equivalent to that of virus isolation in cell culture. However, PCR was shown to be faster and easier to perform and may be a good alternative to virus isolation especially when bovine semen has to be screened for BHV-1 prior to artificial insemination.
Histone-to-protamine exchange causes chromatin condensation ceasing gene expression in elongating spermatids. Gene expression of protamines is regulated by the transcription factor cAMP-responsive element modulator (CREM). Altered CREM expression results in male infertility, as shown by CREM-knock-out mice being sterile due to round spermatid maturation arrest and patients exhibiting round spermatid maturation arrest revealing a lack or substantial reduction of both CREM-mRNA and CREM-protein. Similar defects in histone-to-protamine exchange have been suggested in infertile stallions exhibiting enlarged sperm heads. The CREM-gene consists of 14 exons. Alternative exon splicing results in the production of both activator and repressor proteins. To further clarify the role of different CREM-isoforms for male infertility, the expression pattern of various CREM-isoforms during equine and human normal and impaired spermatogenesis was investigated by RT-PCR. Stallions with normal spermatogenesis expressed six activators and three repressors. In men three activators and seven different repressors were detected. In one stallion and patients with impaired spermatogenesis, only repressors were found. It is concluded that (i) stallion and man reveal a different CREM expression pattern, (ii) the expression of CREM activators is a prerequisite for normal spermatogenesis, and (iii) the lack of CREM activator expression results in male infertility.

The objective of this study was to explore approaches to decontaminate embryos either contaminated naturally or under experimental conditions with different viruses. Embryos were obtained from in vitro maturation and fertilisation of porcine oocytes. After 7 days of development, morula and blastocyst stages were exposed for 1 h to the following viruses: encephalomyocarditis virus (EMCV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), and bovine viral diarrhea virus (BVDV) at an infectivity of 100 TCID50/mL. Embryos samples were treated with different washing procedures, which all included the following standard washing solutions: PBS+0.4% BSA (five times for 10 s), Hank's + 0.1% hyaluronidase (one time for 5 min) instead of trypsin and a pre-incubation with oviductal cells. Therefore, in the first experiment, oocytes received standard maturation treatments and in the second, they were also co-incubated with oviductal cells for the last 3 h of maturation. The effectiveness of the different washing techniques in removing viruses was evaluated by polymerase chain reaction (PCR) analysis. In the first experiment, trypsin treatment did not eliminate PRRSV, PPV, PCV, and EMCV from contaminated embryos. Surprisingly, treatment with hyaluronidase eliminated all tested viruses. In the second experiment, all viruses tested were removed from the oocytes following the different enzymatic treatments. In conclusion, in vitro embryo decontamination was more effective following exposure to oviductal secretions and hyaluronidase eliminated more virions than trypsin in washing techniques.
To improve the knowledge on the risk of transmission of the caprine arthritis-encephalitis virus (CAEV) during embryo manipulations, we conducted a double-nested polymerase chain reaction (PCR) for CAEV proviral-DNA on flushing media recovered from the oviducts 48 h after the beginning of estrus and on blood from 89 donor does. Sixty-four does had negative blood and flushing media by PCR. Among the 25 CAEV infected goats (blood PCR positive), 11 were PCR flushing media positive (PThe mean number of embryos recovered was not significantly different between goats with flushing media PCR positive and goats with flushing media PCR negative (6.0+/=5.4 versus 7.8+/=4.4, respectively: mean+/=S.D.) nor between goats with blood PCR positive and goats with blood PCR negative (7.0+/=5.0 versus 5.9+/=5.3: mean+/=S.D.). The presence of CAEV infected cells in oviductal flushing media from infected donor does was indicated for the first time during this study. The absence of flushing media PCR positive for goat blood PCR negative seemed to allow the use of the blood PCR test to confidently predict the absence of CAEV provirus in the oviductal fluid.

Transmission of caprine arthritis-encephalitis virus (CAEV) is not completely understood and the vertical route of infection from the goat to the embryo or to the fetus needs to be investigated. This route of infection involves the presence of CAEV in the genital tract tissues. Prior studies have detected CAEV-infected cells in genital secretions and in flushing media recovered during embryo collection from infected goats. To specify the origin of these cells, we conducted a double-nested polymerase chain reaction (PCR) test on embryo flushing media and on mammary gland, mammary lymph node, synovial membrane, pelvic lymph node, uterus and oviduct tissues from 25 CAEV-infected (blood PCR positive) embryo donor goats for the presence of CAEV proviral DNA. The presence of proviral DNA was found in 22 of 25 mammary gland samples, 14 of 25 uterus samples, and in 16 of 25 oviduct samples. Nineteen of 25 goats had at least one positive genital tract sample. Flushing media from 11 goats were PCR positive. All goats with positive-flushing media were oviduct positive. Of this group of does, except for 1 of the 11, infection of flushing media correlated with infection of almost all the other tissues examined. The frequency of positive tissues for flushing media-positive goats (61/66; 92%) was significantly higher than that for flushing media-negative goats (50/84; 60%) (PThis study demonstrated the presence of CAEV-infected cells in the goat genital tract. The presence of CAEV-infected cells in the uterus and oviducts suggests potential for vertical transmission of CAEV from doe to embryo or fetus.
To establish the polymerase chain reaction (PCR) method for detecting the XY cells in cases suspected to have the bovine freemartin syndrome, a PCR reaction test was conducted on blood from a normal bull diluted in blood from a normal cow. From the results obtained, it was shown that the Y-specific sequence was detectable down to a concentration of 0.1%. Various types of the bovine freemartin syndrome, which occurs in heterosexual twins, single-born sterile heifers, and heifers born with Acardius amorphus, were examined by the chromosome analysis and the PCR method. The Y-specific sequence was detected in all 26 cases that showed chromosome chimerism but which was absent in the 5 cases without a chimerism. The PCR method was found to be effective and convenient for quickly diagnosing the various types of bovine freemartin syndrome.


http://www.sciencedirect.com/science/article/B6TCM-49PSB4V-BX/2/ac8d75b6f6b522251357ea6aefbde64a

Commonly used reagents in the culture and transfer of embryos are isolated from blood and tissue samples and thus have the potential for chromosomal and or mitochondrial DNA contamination. In this study, we evaluated the results obtained from PCR analysis of bovine trypsin, bovine sera, and bovine albumin precipitates. Bovine sera samples that were tested yielded minor to heavy DNA contamination signals depending on the manufacturer and specific type of sera. Bovine albumin precipitates showed very little DNA contamination or none at all. Bovine trypsin samples yielded moderate DNA contamination signals depending on the ability of the trypsin to be inactivated prior to PCR analysis.


http://www.sciencedirect.com/science/article/B6TCM-4CVVC2S-1/2/3dece4b0253af601c2ee985ae6ce38b5

Pre-determination of the sex of offspring has implications for management and conservation of captive wildlife species, particularly those with single sex-dominated social structures. Our goal is to adapt flow cytometry technology to sort spermatozoa of non-human primate species for use with assisted reproductive technologies. The objectives of this study were to: (i) determine the difference in DNA content between X- and Y-bearing spermatozoa (ii) sort sperm nuclei into X- and Y-enriched samples; and (iii) assess the accuracy of sorting. Spermatozoa were collected from two common marmosets (Callithrix jacchus), seven hamadryas baboons (Papio hamadryas) and two common chimpanzees (Pan troglodytes). Human spermatozoa from one male were used as a control. Sperm nuclei were stained (Hoechst 33342), incubated and analyzed using a high-speed cell sorter. Flow cytometric reanalysis of sorted samples (sort reanalysis, 10,000 events/sample) and fluorescence in situ hybridization (FISH; 500 sperm nuclei/sample) were used to evaluate accuracy of sorting. Based on fluorescence intensity of X- and Y-bearing sperm nuclei, the difference in DNA content between X and Y populations was 4.09 +/- 0.03, 4.20 +/- 0.03, 3.30 +/- 0.01, and 2.97 +/- 0.05%, for marmoset, baboon, chimpanzee and human, respectively. Sort reanalysis and FISH results were similar; combined data revealed high levels of purity for X- and Y-enriched samples (94 +/- 0.9 and 93 +/- 0.8%, 94 +/- 0.7 and 94 +/- 0.5%, 91 +/- 0.9 and 97 +/- 0.6%, 94 +/- 0.6 and 94 +/- 0.9%, for marmoset, baboon, chimpanzee and
human, respectively). These data indicate the potential for high-purity sorting of spermatozoa from non-human primates.


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Leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF), members of the group of hemopoietic cytokines, play a primary role in the control of embryo development and implantation and in the growth of the placenta in humans and mice. Gene expressions of LIF and M-CSF were investigated using quantitative RT-PCR in bovine endometrial tissues during early and mid-pregnancy (Days 16-17, 20-21, 30-36, 48-49 and 74-140) and during the estrous cycle (Days 13-14). Leukemia inhibitory factor and M-CSF genes were expressed in all samples examined. Significant differences were found between the gene expression patterns of LIF and M-CSF. Leukemia inhibitory factor expression level at Days 48-49 was the highest in caruncular endometrium, however, the large variability negated any significant differences. Leukemia inhibitory factor expression levels in intercaruncular endometrium at Days 48-49 and 74-140 of pregnancy were greater than at Days 13-14 of the estrous cycle and at other days of pregnancy. No significant change was recognized in M-CSF expression levels in caruncular endometrium. Macrophage colony stimulating factor expression level in intercaruncular endometrium at Days 74-140 was greater than those of the other samples. These results suggest that LIF and M-CSF are produced in the endometrium and may play different roles in early and mid-pregnancy.


http://www.sciencedirect.com/science/article/B6TCM-4B4S5RN7/2/2eaae34ac1b066f09a5423c563878654

Porcine embryonic germ (EG) cells share common features with porcine embryonic stem (ES) cells, including morphology, alkaline phosphatase activity and capacity for in vitro differentiation. Porcine EG cells are also capable of in vivo development by producing chimeras after blastocyst injection; however, the proportion of injected embryos that yield a chimera and the proportion of cells contributed by the cultured cells in each chimera are too low for practical use in genetic manipulation. Moreover, somatic, but not germ-line chimerism, has been reported from blastocyst injection using porcine ES or EG cells. To test whether efficiency of chimera production from blastocyst injection can be improved upon by changing the host embryo, we used as host embryos four groups according to developmental stage or length in culture: fresh 4-cell and 8-cell stage embryos subsequently cultured into blastocysts, fresh morulae, fresh blastocysts, and cultured blastocysts. Injection and embryo transfer of fresh and cultured blastocysts produced similar percentages of live piglets (17% versus 19%). Four piglets were judged to have a small degree of pigmentation chimerism, but microsatellite analysis failed to confirm chimerism in these or other piglets. Polymerase chain reaction analysis for detection of the porcine SRY gene in female piglets born from embryos injected with male EG cells identified six chimeras, at least one, but not more than two, from each treatment. Chimerism was confirmed in two putative pigmentation chimeras and in four piglets without overt signs of chimerism. The low percentage of injected embryos that yielded a chimera and the small contribution by EG cells to development of each confirmed chimera indicated that procedural changes in how EG cells were combined with host embryos were unsuccessful in increasing the likelihood that porcine EG cells will participate
in embryonic development. Alternatively, our results suggested that improvements are needed in EG cell isolation and culture procedures to ensure in vitro maintenance of EG cell developmental capacity.


http://www.sciencedirect.com/science/article/B6TCM-46SG670-5/2/d82c19ecd4b0991fd4e42175ba07ff6

Behavioral estrus and components of litter size at Day 35/36 of pregnancy were studied in gilts with prolactin receptor (PRLR) genotype AA (n=9), AB (n=25), and BB (n=22). This PRLR polymorphism (two alleles, A and B) has been associated with litter size, although it is not known whether the polymorphism itself causes differences in litter size or whether it is a marker for a closely linked causative gene. Estrus length in three successive estrous cycles was not affected by genotype, but estrous cycle length tended (P<0.05) gilts. Results of this experiment show that the PRLR gene or a very closely linked gene affects porcine ovaries, uterus, and placenta in a way that might lead to differences in litter size. Since other genes and also environmental factors, however, might change the effect within the 112 days to parturition, it is preferable to state that the PRLR gene is a candidate gene for ovulation rate rather than for litter size.


http://www.sciencedirect.com/science/article/B6TCM-44X0BGC-9/2/2cc8807b0cc87967270316dae96cf4a9a

Seventy-seven Large White x Meishan F2 crossbred gilts with prolactin receptor (PRLR) genotype AA (n=26), AB (n=36) and BB (n=15) were compared for teat number (FTm), age at first estrus, gestation length (GL), litter size, and litter means of functional teat number (FTp), birthweight (BW), and pre-weaning growth rate (GR). Own placental information was available for 88% of 620 live-born piglets (62 gilts), since placentae were labeled during farrowing. The effect of PRLR genotype of the mother on average placenta weight (PLW) and placenta efficiency (EFF=BW/PLW), was therefore, also analyzed. PRLR genotype significantly affected (P<0.056) number of piglets born alive (NBA, P=0.072), but it did not affect (P>0.3) GL, BW or GR, before nor after correction for litter size. BB gilts were significantly younger at first estrus and younger and lighter at insemination than AA gilts (P=0.047) and tended to have a larger NBA (P=0.062) than BB gilts. TNB was 11.4+-0.7, 10.8+-0.6, and 8.8+-0.9; NBA was 11.1+-0.6, 10.5+-0.6, and 8.7+-0.9; BW was 1309+-40, 1277+-34, and 1290+-53 g; and GL was 113.6+-0.3, 113.8+-0.3, and 113.5+-0.4 days for AA, AB and BB gilts, respectively. The effects on litter size and age at first estrus are independent effects. PRLR affected PLW (P=0.050) and EFF (P=0.066), resulting in a difference between AA and BB gilts. PLW was 160+-9, 181+-7 and 196+-11 g and EFF was 7.6+-0.2, 7.3+-0.2 and 6.7+-0.3 for AA (n=19), AB (n=29) and BB (n=14) gilts, respectively. After correction for TNB, the differences disappeared. Functional teat number of the AA, AB and BB gilts was 15.35+-0.22, 15.53+-0.18, and 15.60+-0.29, respectively, and was not affected by PRLR genotype (P=0.7). Functional teat number of piglets from AA, AB and BB mothers was 14.20+-0.10, 14.37+-0.08, and 14.63+-0.13, respectively. Piglets from BB mothers had on average larger numbers of functional teats compared to piglets from AA mothers (P=0.028). In conclusion, PRLR gene is a major gene or marker for age at first estrus, litter size, and litter average of number of functional teats in the Large White x Meishan F2 crossbred gilts studied. The favorable allele for litter size (A allele) is the unfavorable allele for...
age at first estrus and for litter mean of functional teat number.

Thorax (3)


http://thorax.bmjournals.com/cgi/content/abstract/57/9/765

Background: The use of reverse transcription-polymerase chain reaction (RT-PCR) to measure mRNA levels has led to the common use of {beta}-actin and GAPDH housekeeping genes as denominators for comparison of samples. Expression of these genes is assumed to remain constant, so normalising for variations in processing and signal quantitation. However, it is well documented that {beta}-actin and GAPDH expression is upregulated with proliferation, activation, and differentiation. We hypothesised that airway samples which differ in their cellular profiles and activation status have different levels of expression of GAPDH and {beta}-actin. Methods: The mRNA for {beta}-actin, GAPDH, and interleukin (IL)-2 was measured in bronchoalveolar lavage (BAL) fluid cells and endobronchial biopsy tissue by competitive RT-PCR in a cross sectional study of 26 normal controls and 92 asthmatic subjects. Results: For both BAL fluid cells and biopsy tissue, asthmatics overall had reduced expression of GAPDH and {beta}-actin mRNA. In asthmatic subjects not using inhaled corticosteroids (ICS), GAPDH mRNA levels in both BAL fluid and biopsy tissue, and {beta}-actin mRNA in BAL fluid cells were 10 times lower than samples from both normal controls and from asthmatic subjects using ICS. {beta}-Actin mRNA in biopsy specimens showed the same pattern of expression, but asthmatic subjects not using ICS were not significantly different from those receiving ICS treatment. IL-2 mRNA levels did not differ between the subject or treatment groups but, when expressed as a ratio with {beta}-actin, significant differences were seen. Conclusions: {beta}-Actin and GAPDH used as denominators of gene expression quantitation in asthma research can cause confounding. Housekeeping genes need careful validation before their use in such quantitative mRNA assays.


http://thorax.bmjournals.com/cgi/content/abstract/58/9/772

Background: Subepithelial collagen and extracellular matrix protein deposition are important pathophysiological components of airway remodelling in chronic asthma. Animal models based on the local reaction to antigens show structural alterations in the airway submucosal region and provide important information regarding disease pathophysiology. We describe a murine model of peribronchial fibrosis using intratracheally instilled transforming growth factor (TGF)-{beta}1 in BALB/C mice that facilitates a mechanistic approach to understanding the cellular and molecular pathways leading to airway fibrosis. Methods: BALB/C mice were intratracheally instilled with either TGF-{beta}1 or buffered saline. Airway fibrosis was assessed by light microscopy, hydroxyproline content, and polymerase chain reaction (PCR) for collagen I and III on microdissected airway samples. The lysyl oxidase inhibitor {beta}-aminoproprionitrile (BAPN) was administered to TGF-{beta}1 treated mice to block airway collagen deposition. Airway
hyperresponsiveness was also measured after treatment with TGF-{beta}1. Results: During the 7
days after administration of TGF-{beta}1 the mice developed increased subepithelial collagen
which could be blocked by BAPN. Increased mRNAs for collagen types I and III were seen in
microdissected airways 1 week after TGF-{beta}1, and significantly increased total collagen was
found in the airways 4 weeks after TGF-{beta}1. A detectable increase in airway hyperreactivity
occurred. Conclusions: This new model should facilitate detailed study of airway remodelling that
occurs in the absence of detectable cellular inflammation, and allow examination of the functional
consequences of a major structural alteration in the conducting airways uncomplicated by
inflammatory cell influx.

Tanino, M., T. Betsuyaku, et al. (2002). "Increased levels of interleukin-8 in BAL fluid from smokers
susceptible to pulmonary emphysema." Thorax 57(5): 405-411.

http://thorax.bmjournals.com/cgi/content/abstract/57/5/405

Background: It has previously been shown that smokers with computed tomographic (CT)
evidence of subclinical emphysema have signs of neutrophil activation, despite having no
appreciable increase in the number of neutrophils in their bronchoalveolar lavage (BAL) fluid.
Methods: The levels of the following chemoattractants in BAL fluid from 61 community based
older volunteers classified into four groups according to current smoking status and the presence
or absence of emphysema were determined: interleukin 8 (IL-8), epithelial neutrophil activating
protein 78 (ENA-78) and leukotriene B4 (LTB4) which are primarily chemotactic for neutrophils;
monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein-1{alpha}
(MIP-1{alpha}) which are predominantly chemotactic for mononuclear leucocytes. Results: Of the
five chemoattractants studied, only the level of IL-8 in BAL fluid clearly distinguished between
subjects with and without emphysema among current smokers (median values 34.7 and 12.2
pg/ml, respectively, p<0.01). In addition, the levels of IL-8 and neutrophil elastase-{alpha}1
protease inhibitor complex in BAL fluid were significantly correlated (r=0.65, p<0.01). There was
no difference in either the release of IL-8 from cultured alveolar macrophages at 24 hours or the
expression of IL-8 messenger RNA of alveolar macrophages in the two groups of current
smokers with and without emphysema. Conclusion: An accelerated response of IL-8 to chronic
smoking is a factor that characterises those smokers who are susceptible to pulmonary
emphysema, although the cellular source of IL-8 remains to be determined.

Thrombosis Research (24)


http://www.sciencedirect.com/science/article/B6T1C-4096410-6/2/b4022aa8f8d898804f76f4de171f931b

It has been shown recently that the variable expression of the platelet collagen receptor integrin
[alpha]2[beta]1 predisposes to thrombotic risk on the one hand and hemorrhagic risk on the other
hand. The level of expression of the integrin [alpha]2[beta]1 is genetically controlled and
associated with the [alpha]2-807 dimorphism. The expression level of this platelet collagen
receptor may play a central role in the rapidly evolving coronary artery lesions that lead to
malignant arrhythmia and sudden cardiac death. We studied allelic frequencies of the [alpha]2-807 dimorphism for their relation as a risk factor for malignant arrhythmia in a well-defined subgroup of patients with coronary artery disease. We compared allelic frequencies (by sequence specific primer polymerase chain reaction) of the dimorphism that is associated with integrin [alpha][2][beta][1] levels in 94 Caucasoid survivors of sudden cardiac death with a matched group of 106 patients with coronary artery disease without sudden cardiac death. Gene frequencies in the patient groups did not differ and were similar to those in the general population represented by 217 healthy individuals. There was no overrepresentation of an allele in any group. The inherited dimorphism that is associated with the levels of platelet integrin [alpha][2][beta][1] is not associated with malignant arrhythmia in coronary artery disease patients.


http://www.sciencedirect.com/science/article/B6T1C-3RD1C85-4/2/9a614d671ed73585ae4fcfe7152147

Precise determination of mRNA levels is an essential element in any investigation of complex regulatory systems. Classical methodologies such as Northern hybridization suffer from requirements for significant samples of material and also a degree of non-specificity. Recently, quantitative techniques involving PCR amplification have been devised. We have developed and applied such procedures to the determination of prothrombin messages in skeletal muscle cells during development. In addition to its role in the blood coagulation cascade, the serine protease thrombin has been shown to participate in several signaling events in the neuromuscular system. The inactive precursor, prothrombin, primarily produced in the liver, has also been shown to be synthesized and developmentally-regulated in the brain. In skeletal muscle, thrombin is a mediator of activity-dependent polynuclear synapse elimination (ADPSE) which occurs in early postnatal development. Recent experiments showing that thrombin is released from myotubes in culture under the influence of acetylcholine suggest that locally-synthesized prothrombin may be the source of this Hebbian synaptic interaction. We have determined that prothrombin is expressed in skeletal muscle, as the likely source of thrombin involved in ADPSE, and the current results show the quantitative expression of muscle prothrombin during this time of intense synapse remodeling. Published by Elsevier Science Ltd


http://www.sciencedirect.com/science/article/B6T1C-41185GV-2/2/1fbf20f2c49819f8e726a49b321b47d0

The frequencies of Factor V G1691A (FVL), prothrombin (PT) G20210A, 5’10’-methylene tetrahydrofolate reductase (MTHFR) C677T, and methionine synthase (MS) A2756G (four mutations associated with an increased risk of venous thromboembolism [VTE]) were determined in a sample of approximately 1500 New York State residents. Dried blood spots from approximately equal numbers of Caucasians, African-Americans and Hispanics were anonymously obtained from the New York State Department of Health Newborn Screening Program. Following PCR amplification of dried blood spot DNA, allele-specific oligonucleotide hybridization was used to detect mutant alleles. The total number of individuals at increased genetic risk for VTE was 271 (17.5%) of the 1553 persons tested. Increased genetic risk was defined as the heterozygous state for FVL or PT and the homozygous state for the MTHFR or MS
Sixteen individuals had more than one genetic risk factor. The MS gene variant allele frequencies for African-Americans and Hispanics are the first to be reported. This report also provides an estimate of the variant PT allele in the largest group of Hispanics studied to date.


http://www.sciencedirect.com/science/article/B6T1C-4B4YW7W-3/2/d5c1733691a0d2720ef4d4028561876f


http://www.sciencedirect.com/science/article/B6T1C-42HFNP9-5/2/09dd3f777e37fb1de517b1efd0877113

Coagulation factor XII (FXII) deficiency is rarely found to be associated with bleeding, but single reports demonstrated thromboembolic events in FXII-deficient patients. Currently, the biological role of FXII is still discussed controversially. It is well known that plasma levels of FXII show great interindividual variability. Recently, it has been demonstrated that a frequently occurring C->T polymorphism in the FXII promoter region at nucleotide (nt) 46 is associated with lower plasma FXII activity levels in Orientals. In our study, we evaluated the frequency of this polymorphism in a randomly selected sample of newborns and investigated whether this C->T polymorphism also contributes to the frequently observed moderate FXII deficiency in Europeans. We developed a new mutagenically separated polymerase chain reaction assay (MS PCR), which allows mutation detection without the use of restriction enzymes. Among 100 healthy newborns, we found 64% homozygous carriers of the wildtype FXII 46C allele, 29% were heterozygous for FXII C46T, and 7% homozygous for FXII 46T. Evaluation of plasma FXII activity and genotype in 80 randomly selected and unrelated individuals revealed a highly statistically significant (P<.001) association of the FXII 46T allele with reduced FXII plasma activity. Individuals carrying the homozygous FXII 46C genotype had a mean of 1.17 U/ml (+/-0.31 U/ml), individuals heterozygous for FXII C46T showed a mean of 0.70 U/ml (+/-0.31 U/ml), and subjects homozygous for FXII 46T had only 0.44 U/ml (+/-0.10 U/ml) plasma FXII activity.


http://www.sciencedirect.com/science/article/B6T1C-4CBVKT4-1/2/8cc57bc9bab0d3a76aaa24c7cb077a54

Heme oxygenase-1 (HO-1) has been demonstrated to exert potent anti-oxidant and anti-inflammatory effects in the context of atherosclerotic vascular disease, and therefore was referred to as a potential vascular protective factor. A (GT)n dinucleotide repeat polymorphism in the HO-1 promoter has been shown to modulate HO-1 gene expression. Short (We determined the number of GT repeats in the HO-1 promoter in 399 patients with ischaemic cerebrovascular events and 398 healthy controls and compared the frequencies of short (=25) repeat (class L) alleles after
adjustment for potentially confounding factors. Genotype distributions of S/S, S/L and L/L in patients were 9.8% (n=39), 45.1% (n=180) and 45.1% (n=180), which was similar to the distribution in controls with 11.5% (n=46), 44.5% (n=177) and 44.0% (n=175). In the presence of vascular risk factors, the HO-1 genotype became functionally relevant: in patients without hyperlipidemia the S/S genotype exerted a protective effect on the development of ischaemic cerebrovascular events (OR 0.2; 95% CI 0.1-0.6), while this effect was no longer present in hyperlipidemic patients. Short (<25 GT) repeats in the HO-1 gene promoter confer a reduced risk for cerebrovascular events in individuals with normal plasma lipid levels. This may explain controversial findings in different populations.


http://www.sciencedirect.com/science/article/B6T1C-3V4X5FX-5/2/d5c2b2bb991aefd9b8e042948663bce7


http://www.sciencedirect.com/science/article/B6T1C-3VWNB97-P/2/c6fe5cbb115d2257e0504a215d562ec8


http://www.sciencedirect.com/science/article/B6T1C-3RH1FTJ-6/2/cfe840d9374078de01f327e804637d49

Plasma plasminogen activator inhibitor-1 (PAI-1) level was observed to be associated with sequence variations at the PAI-1 locus. Therefore, PAI-1 gene promoter was screened for possibly new polymorphisms and to investigate the contribution of these sequence variations to PAI-1 levels in patients with deep vein thrombosis (DVT). DNA was isolated from blood of 83 consecutive unrelated patients (42+/-11 years old) and from 50 apparently healthy subjects of similar age and gender distribution. Six fragments covering DNA sequence - 1523 base pairs (bp) upstream from the start of PAI-1 gene transcription to +90 bp in the first exon, were amplified by polymerase chain reaction and analyzed by single-strand conformation polymorphisms. Two polymorphisms were found: a previously described 4G/5G deletion/insertion polymorphism - 675 bp upstream from the start of transcription and a novel G/A single base substitution polymorphism further upstream at -844 bp. The two polymorphisms were in strong linkage disequilibrium. Significant differences between patients and controls were observed neither for the frequencies of the 4G/5G alleles (and, respectively) nor for the frequencies of the G/A alleles (and, respectively). The distribution of both polymorphisms was similar in idiopathic and secondary DVT as well as in first and recurrent DVT. In patients association between the 4G/5G genotypes and PAI activity was observed, with the highest values in the 4G/4G genotype (13.3 U/mL), median values in the 4G/5G genotype (9.8 U/mL) and the lowest values in the 5G/5G genotype (2.0 U/mL). Despite the lack of association between the G/A genotypes and plasma PAI-1 levels, electrophoretic mobility
shift assay showed specific binding of a nuclear protein from human vascular endothelial cells extracts to both the G and the A variant, suggesting functional importance of this novel G/A polymorphism in regulating the expression of PAI-1 gene. Copyright (c) 1996 Elsevier Science Ltd


http://www.sciencedirect.com/science/article/B6T1C-46HFP67-5/2/75be73ed3a4aaef7696ee19907756a50

Fasting plasma homocysteine level and the related clinical findings were analysed in 240 consecutive patients with venous thromboembolism. Hyperhomocysteinemia, defined as a plasma level above 20 [mu]mol/l (corresponding to the percentile 95th in the controls), was present in 11.2% of the patients. Plasma homocysteine level was similar in patients presenting with either deep venous thrombosis, pulmonary embolism or both conditions. It was significantly higher in patients with primary (unprovoked) VTE than in patients with secondary disease (associated with at least one risk factor): 12.3 vs. 9.55 [mu]mol/l (ppp=0.034). Furthermore, hyperhomocysteinemia was correlated with reduced protein C level (p=0.013). In a multivariate analysis, two factors were significantly associated with hyperhomocysteinemia: older age (pp<0.02). Since the frequency of homozygous MTHFR thermolabile variant was rather similar in patients and controls, testing for C677T mutation was not helpful in screening VTE patients. However, the homozygous mutation was significantly more prevalent among hyperhomocysteinemia patients, confirming its role in the genesis of hyperhomocysteinemia. According to its prevalence, to the putative role in venous and arterial disease and the availability of an effective and low-cost corrective therapy, hyperhomocysteinemia deserves interest, especially in the elderly and in the patients with idiopathic VTE disease.


http://www.sciencedirect.com/science/article/B6T1C-4B8X378-2/2/8ba5a2dd652fa8507d2ad0bb1268c30

Introduction: Astrocytes are known to regulate a wide variety of brain endothelial cell functions. Prior work, using a mixed species co-culture system, has shown astrocyte regulation of brain capillary endothelial expression of key hemostasis factors tissue plasminogen activator (tPA) and its inhibitor, plasminogen activator inhibitor-1 (PAI-1). The purpose of this study is to define the fibrinolytic regulatory role of human astrocytes on human brain capillary endothelial cells.

Materials and methods: We used a blood-brain barrier model consisting of human astrocytes grown on transwell membrane inserts and co-cultured with human brain capillary endothelial cells. Following 48 h co-culture, we analyzed both endothelial mono-cultures and astrocyte-endothelial co-cultures for expression of tPA and PAI-1 mRNA, protein, and activity. Results and conclusions: There were significant changes for both tPA and PAI-1 mRNA. tPA mRNA levels were decreased in co-cultures (55+/-16% of mono-cultures, pppp<0.0005). TGF-[beta] neutralizing antibody attenuated the observed changes in both tPA and PAI-1. These data indicate that human astrocytes regulate human brain capillary fibrinolysis in vitro by inhibiting tPA and enhancing PAI-1 expression. This regulation is mediated, in part, by transforming growth factor-[beta]. Our findings provide further evidence for the role of astrocytes in brain-specific hemostasis regulation.
Platelet glycoprotein (GP) Ib/IX/V complex is a major receptor for von Willebrand factor (vWF), which mediates platelet adhesion and aggregation under high shear stress conditions. It is composed of GPIb[alpha], GPIb[beta], GPIX, and GPV. All subunits for the human receptor have been cloned and characterized. However, the function of GPIb[beta] is still elusive. To obtain further information of GPIb[beta], we have determined the genomic sequence of mouse GPIb[beta] (1466 bp). The deduced amino acid sequence (206aa) was 88% identical to the human GPIb[beta] protein. All cysteine residues, putative N-linked glycosylation site (Asn41), and putative phosphorylation site (Ser166) were conserved. The promoter region contained putative GATA and ets binding motif implicated in megakaryocytic expression. Mouse GPIb[beta] also contained a leucine-rich glycoprotein (LRG) sequence of 24 amino acids same as human GPIb[beta].

Resistance to activated protein C (APC) is the most prevalent single phenomenon associated with thromboembolic disease. It is caused by a single point mutation in the factor V gene (Arg506Gln or FV Leiden), replacing an Arg506 with a Gln at the APC-cleavage site in factor V. In this study we present a prevalence study of the Arg506Gln mutation in a large Danish cohort. By screening 4188 newborns (8376 alleles) we identified 3.4% alleles (95% CI: 3.0-3.8) of the Arg506Gln mutation, corresponding to a heterozygous prevalence of 6.6% (95% CI: 5.9-7.4) in Denmark. This is significantly lower than what has been reported from southern Sweden. The birth cohort has been selected from the entire country, providing representative and accurate estimates of the gene frequencies. Equal gender distribution was found, and the Arg506Gln mutation is probably not a considerable risk factor in fetal life in the general population.

The cDNA sequence of mouse protein S was derived by conventional PCR amplification from liver mRNA, initially using primers derived from the human cDNA sequence, followed by direct DNA sequencing. Seven overlapping PCR fragments covering all of the mature protein, part of the propeptide, and the 3' noncoding region were generated and sequenced. In some cases primers based upon the human cDNA sequence were ineffective. Subsequent successful amplification with mouse-derived primers to the same regions and comparison of the mouse and human sequences in these regions suggest that the failure of the human primers was due to insufficient degree of heterospecies identity. The mouse protein S cDNA sequence of the coding
region shares 82% identity to human. The 3’ noncoding region of mouse protein S cDNA has several small deletions and insertions compared to human protein S cDNA. Mature mouse protein S consists of 634 amino acids in a single polypeptide chain and displays domain organization similar to that for other species. The amino acid sequence of mouse protein S is about 80% identical to that of other species. Eleven glutamic acid residues were found in the amino terminal region and are predicted to be sites of [gamma]-carboxylation. Amino acid residues #80244 are defined as four cysteine-rich repeat sequences homologous to epidermal growth factor. The remainder of the molecule is homologous to plasma sex steroid binding protein. The mouse protein S contains two potential N-glycosylation sites at positions #458 and 468 and is lacking the putative glycosylation site at #490 found in human protein S.


http://www.sciencedirect.com/science/article/B6T1C-4BDM1XH-1/2/230d9df3b18e0940ecdbb8063cba69b9

Background: Tissue factor (TF) is primarily known for its function to initiate blood coagulation. The range of in vivo expression of TF is wide and requires a dynamic assay for monitoring. A general method for TF mRNA quantitation that is dynamic, sensitive and applicable to a variety of experimental systems or clinical situations is therefore desirable. Objectives: To develop a method for sensitive and dynamic quantitation of TF mRNA in human blood cells. Methods: TF mRNA expression was analysed and evaluated in monocyte isolations, in whole blood (healthy volunteers and patients scheduled for percutaneous coronary intervention, PCI) and in a panel of human cell lines. RNA was extracted, reverse transcribed and subjected to real-time PCR amplification, according to the TaqMan technology. A TF plasmid was constructed as calibrator of the assay. Two housekeeping genes used as endogenous controls for cDNA quality and integrity were evaluated. Results: The assay was linear by seven orders of magnitude and detected down to 102 copies of the TF plasmid. The coefficient of variation was 4% intra-assay and 28% between the assays when using [beta]2MG as endogenous control. The [beta]-actin gene expression was induced by treatment with lipopolysaccharide (LPS) in blood leukocytes and could not be used as an endogenous control. However, [beta]2MG showed only minor variations upon treatment with LPS. The TF mRNA and antigen expression, measured in a Western blot, correlated well (R2=0.903) in a panel of 11 human cell lines. Conclusions: We have established a method for sensitive and dynamic quantitation of TF mRNA in experimental systems and for clinical situations.


http://www.sciencedirect.com/science/article/B6T1C-430NR3N-C/2/66140550144752a35abe142253513cc3

In the present study, we have shown that stimulation of cryopreserved, human peripheral blood monocytes with the cell wall components from Gram-negative bacteria, lipopolysaccharide (LPS), and from rapid-growing Mycobacterium sp., non-mannose-capped lipoarabinomannan (AraLAM), both induce expression of the "early immediate genes" tissue factor (TF) and tumor necrosis factor-[alpha] (TNF-[alpha]). This was demonstrated both at the protein and the mRNA levels. Antibodies against the CD14 receptor could block the stimulating effects. AraLAM was a significantly weaker inducer than LPS, and we speculate that this may reside in the number of the fatty acids in the part of the molecule that interacts with the CD14/Toll-like receptors (TLR).
Finally, both LPS and AraLAM activated the "early immediate genes" through translocation of the transcription factor proteins NF-κB/Rel and increasing the binding activity of AP-1.


http://www.sciencedirect.com/science/article/B6T1C-49NVDS7-1/2/54f35f6b16adc9b05d68507ffbc6c6db8

Aim: The purpose of the study is to investigate whether hypercoagulation in diabetes is observed not only in increased plasma levels of the coagulative factors but also in increased hepatic mRNA levels. Materials and methods: The age-related changes of coagulation factors were determined using KK and KK-Ay mice as a model for human type 2 diabetes mellitus. Expression of the [alpha]-, [beta]- and [gamma]-chains of fibrinogen-mRNA and prothrombin-mRNA from the mouse liver was examined by reverse transcription-polymerase chain reaction (RT-PCR). Results: Hemoglobin A1c (HbA1c), plasma fibrinogen, triglyceride and insulin levels increased apparently, especially in KK-Ay mouse at 4 months old. The mRNA levels of [gamma]-chain of fibrinogen significantly increased at 3 and 4 months of age in both mice. The mRNA levels of [alpha]- and [beta]-chains of fibrinogen and prothrombin were significantly increased in 4-month-old KK-Ay mouse. Conclusions: These results suggest that the increase in hepatic mRNA expression of coagulant factors contributes to the hypercoagulable state in type 2 diabetic mice.


http://www.sciencedirect.com/science/article/B6T1C-42HFNP9-1/2/6e1209c8cd2586ab51ce811ee8208756

Low levels of factor X (F.X) were detected in a 4-year-old boy who experienced acute lymphoblastic leukemia and bleeding manifestations. Laboratory data suggested the presence of a dysfunctional F.X molecule. Two novel F.X gene mutations were identified in the proband that was double heterozygous for both: a microdeletion (delC556) in exon VI resulting in a frameshift leading to a termination codon at position 226. This deletion was found in six family members with reduced F.X antigen and activity levels. A second mutation characterised by a G1344->C transversion in exon VIII was detected in the proband resulting in a Lys408->Asn substitution. This latter mutation was present in several asymptomatic family members from the paternal and the maternal side. The proband's sister was homozygous for the Lys408->Asn substitution and exhibited low F.X activity with a normal antigen level. The naturally occurring F.X Lys408->Asn (F.XK408N) variant was isolated from plasma of either homozygous or double heterozygous individuals. NH2-terminal sequencing of the heavy chain of F.XK408N failed to show any sequence abnormality in patients who were also carriers of the delC556, suggesting that this latter lesion accounted for the lack of F.X synthesis. Purified F.X Lys408->Asn had an identical behaviour to normal F.X as judged by SDS-PAGE and immunoblotting. Clotting assay using purified F.XK408N and F.X-deficient plasma resulted in a laboratory phenotype similar to that observed in a homozygous subject for F.X Lys408->Asn substitution. This is the first characterisation of a naturally occurring F.X variant with a mutation at the COOH-terminal end of the molecule.
Warfarin, an antagonist of vitamin K, causes diminution of vitamin K-dependent coagulation factors in the circulation. Although all vitamin K-dependent factors have Gla domains, the warfarin-induced decrease in their plasma concentration differs among factors. In warfarin-treated HepG2 cells, we found modest and severe intracellular degradation of prothrombin and protein C, respectively. To investigate the structural features of these proteins that contribute to their warfarin sensitivity, chimeric prothrombin containing the prepropeptide and Gla domain of protein C was expressed in baby hamster kidney (BHK) cells. This chimera showed similar secretion kinetics and warfarin sensitivity to those of wild-type prothrombin, demonstrating that the Gla domain cannot solely explain the warfarin sensitivity of protein C. In contrast, two chimeric protein Cs containing either the Gla domain alone or the prepropeptide and Gla domain of prothrombin showed impaired secretion. Even though [gamma]-carboxylation proceeded normally, both chimeras were degraded intracellularly by the proteasome. From these results, we conclude that not only the folding of the Gla domain, but the entire structure and conformation of protein C and prothrombin, contribute to their quality control and susceptibility to warfarin-induced ER (endoplasmic reticulum)-associated degradation.


 We report a novel mutation in Factor X (FX) gene which results in a phenotype without any bleeding tendency. The proband has been found to be a compound heterozygote between a novel FX true deficiency (Gly380->Arg) and a previously reported dysfunctional mutation Ser334->Pro (FX Marsiglia). Prothrombin time (PT) and partial thromboplastin time (PTT) were moderately prolonged and were fully corrected by the addition of normal serum. Her FX activity level varied between 8% and 19% of normal according to the method used whereas the FX antigen level was 40% of the normal control value. All the exons and intron/exon junctions of the FX gene were studied using a combined approach of polymerase chain reaction and conformation sensitive gel electrophoresis. A transversion G to A in exon 8 resulting in the replacement of Gly380 by Arg was found in the proband, in the father and in a proband's brother, whereas heterozygous FX Marsigilia was present in the proband's mother and her sister. Gly380 is strictly linked to Ser379, a component of the catalytic triad. The substitution of Gly for Arg causes the introduction of a large charged amino acid which could affect the catalytic function of FX leading to secretion problem, accounting for the cross-reactive material (CRM) negative phenotype.

http://www.sciencedirect.com/science/article/B6T1C-46X91V1-1/2/2d6b61127c97c8fde2f7422e4d5f4f67

Fifteen patients from five families with laboratory data suggesting factor X (FX) deficiency were screened for causative mutations by conformation sensitive gel electrophoresis (CSGE) followed by sequencing. All exonic and flanking intronic regions of factor X gene were amplified using PCR. After heteroduplex formation, samples were analyzed onto a polyacrylamide gel for possible mismatch. An abnormal CSGE profile indicating an heteroduplex was identified in 10/15 cases. All the 10 patients with a patter of migration suggesting a mismatch had a laboratoristic pattern of FX deficiency whereas the five cases with a normal CSGE aspect referred to the normal components of the families who did not carry any FX defect. Sequencing demonstrated that the 10 exons, which showed a suspect CSGE pattern, harbored a mutation responsible for the factor X defect. Of the five mutation identified, two were recognized to be novel mutations (a 871C>T substitution and a 1169G>T transversion in exon 8), both located in the catalytic portion of FX. CSGE may be an effective and simple procedure for screening factor X gene mutations.


http://www.sciencedirect.com/science/article/B6T1C-3R867FF-3/2/405f968602a8ac01ae33045d57b30fa2

A number of genetic risk factors for the development of coronary heart disease has been identified in the past. Some of these represent polymorphisms in genes of proteins which are associated with the process of blood clotting. We investigated the distribution of a recently described G/A polymorphism in the 3'untranslated region of the human prothrombin gene (nt 20210) in 98 patients (19 female age: 53+12 SD years and 79 male, age: 49+8.5 SD years) with coronary heart disease and in 102 healthy newborns by enzymatic amplification of the genomic DNA carrying the polymorphic site and by subsequent restriction digest. The diagnosis of coronary heart disease was established by coronary angiography in all patients. The frequency of the A allele in the healthy newborns was 0.98 % (0.2 % - 3.5 %; CI 0.95) with the G/A genotype occurring in 1.96% (0.24 % - 6.9 %; CI 0.95).In the group of patients with coronary heart disease the G/A genotype was found in 5.1 % (1.7 % - 11.4 %; CI 0.95). 94.9 % of the patients studied showed a G/G genotype. The A/A genotype was neither detected in the newborns nor in the patients with coronary heart disease. This preliminary study strongly suggest that the presence of the G/A polymorphism in the 3'untranslated region of the gene coding for human prothrombin is associated with the occurrence of coronary heart disease. (c) 1997 Elsevier Science Ltd


http://www.sciencedirect.com/science/article/B6T1C-3VXHGFV-M/2/c5b4494088cf39f0f924c38d2c23c468f8

We have successfully developed a murine antifibrin monoclonal antibody designated SZ-63 with
property of binding specifically with thrombus both in vitro and in vivo. In order to reduce its immunogenicity and molecular weight, a murine/human chimeric Fab fragment of the antibody was prepared. mRNA was selected on oligo (dT) cellulose from total RNA isolated from SZ-63 hybridoma cells. cDNAs coding for heavy and light variable regions were amplified by reverse transcriptase polymerase chain reaction. The amplified fragments were cloned and sequenced. The nucleotides of SZ-63 VH and VL are 354 and 321 respectively. The variable genes were then linked with human IgG [gamma]1 CH and [kappa] CL genes. Expression vector pHEN1-63 Fab/Hu was thereby constructed and chimeric Fab fragment was expressed in E. coli HB2151 cells in soluble form. Western blot and ELISA results showed that it remained the same capability of binding with cross-linked fibrin as the murine SZ-63 antibody, the content in culture is about 125 ug/L.

Toxicol. Sci. (15)


http://toxsci.oupjournals.org/cgi/content/abstract/79/2/224

The organo(thio)phosphate esters are one of the most widely used classes of insecticides. Worldwide, organophosphate insecticides (OPs) result in numerous poisonings each year. In insects, glutathione S-transferases (GSTs) play an important role in OP resistance; limited data suggest that GST-mediated O-dealkylation occurs in humans as well. To characterize the capacity of mammalian GSTs to detoxify OPs, we investigated mammalian GST biotransformation of the widely used OP, methyl parathion (MeP). Cytosolic fractions isolated from rat, mouse, and ten individual adult human livers biotransformed 300 (micro)M MeP at rates of 2.36, 1.76, and 0.70 (mean rate) nmol desmethyl parathion/min/mg, respectively. Our study focused on human GSTs; in particular, we investigated hGSTs M1-1 and T1-1, since deletion polymorphisms occur commonly in these genes. However, we found no correlation between hGSTM1/T1 genotypes and MeP O-dealkylation activities of the ten human liver cytosolic samples. We also measured MeP O-dealkylation activities of several purified recombinant GSTs belonging to the alpha (human GSTs A1-1 and A2-2, mouse GSTA3-3, rat GSTA5-5), mu (human GSTs M1a-1a, M2-2, M3-3, M4-4), pi (human GSTP1-1, mouse GSTs P1-1, P2-2), and theta (human GSTT1-1) classes. At 1 mM glutathione and 300 (micro)M MeP concentrations, hGSTT1-1 and hGSTA1-1 exhibited the highest O-dealkylation activities: 545.8 and 65.0 nmol/min/mg, respectively. When expression level and enzymatic activity are considered, we estimate that hGSTA1-1 is responsible for the majority of MeP O-dealkylation in human hepatic cytosol. In target organs such as brain and skeletal muscle, where hGSTT1-1 is expressed, hGSTT1-1-mediated biotransformation of MeP may be important.


http://toxsci.oupjournals.org/cgi/content/abstract/75/2/321

Benzene, a carcinogen that induces chromosomal breaks, is strongly associated with leukemias in humans. Possible genetic determinants of benzene susceptibility include proteins involved in
repair of benzene-induced DNA damage. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), encoded by Prkdc, is one such protein. DNA-PKcs is involved in the nonhomologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair. Here we compared the toxic effects of benzene on mice (C57BL/6 and 129/Sv) homozygous for the wild-type Prkdc allele and mice (129/SvJ) homozygous for a Prkdc functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. Male and female mice were exposed to 0, 10, 50, or 100 ppm benzene for 6 h/d, 5 d/week for 2 weeks. Male mice were more susceptible to benzene toxicity compared with females. Hematotoxicity was evident in all male mice but was not seen in female mice. We observed similar, large increases in both micronucleated erythrocyte populations in all male mice. Female mice had smaller but significant increases in micronucleated cells. The p53-dependent response was induced in all strains and genders of mice following benzene exposure, as indicated by an increase in p21 mRNA levels in bone marrow that frequently corresponded with cell cycle arrest in G2/M. Prkdc does not appear to be a significant genetic susceptibility factor for acute benzene toxicity. Moreover, the role of NHEJ, mediated by DNA-PK, in restoring genomic integrity following benzene-induced DSB remains equivocal.


http://toxsci.oupjournals.org/cgi/content/abstract/79/2/315

Allergic airway diseases induced by low molecular weight (LMW) chemicals, including trimellitic anhydride (TMA), are characterized by airway mucus hypersecretion and an infiltration of eosinophils and lymphocytes. Many experimental models have linked LMW chemical-induced allergic airway disease to Th2 cytokines. Most murine models, however, use dermal exposure to sensitize mice. The present study was designed to test the hypothesis that intranasal sensitization and challenge with the known chemical respiratory allergen TMA, but not the nonrespiratory sensitizers dinitrochlorobenzene (DNCB) and oxazolone (OXA), will induce characteristic features of LMW chemical-induced allergic airway disease in the nasal and pulmonary airways. A/J mice were intranasally sensitized and challenged with TMA, DNCB, or OXA. Only mice that were intranasally sensitized and challenged with TMA had a marked allergic rhinitis with an influx of eosinophils, lymphocytes, and plasma cells, increased intraepithelial mucusubstances, and a regenerative hyperplasia. Cytokine mRNA levels in the nasal airway of TMA treated mice also revealed an increase in the mRNA levels of the Th2 cytokines IL-4, IL-5, and IL-13, but no change in the level of the Th1 cytokine IFN-(gamma). No lesions were found in the nasal airways of mice exposed to DNCB or OXA. TMA increased lung-derived IL-5 mRNA while DNCB and OXA caused no change in lung-derived cytokine mRNA levels. Both TMA and DNCB caused increases in total serum IgE, unlike OXA-exposed mice. However, no adverse alterations were found microscopically in the lungs of mice treated with TMA, DNCB, or OXA. This study is the first to demonstrate that intranasal administration of a known chemical respiratory allergen is an effective method of sensitization resulting in the hallmark features of allergic rhinitis after challenge with a concomitant increase in nasal airway-derived Th2 cytokine mRNA, lung-derived IL-5 mRNA, and total serum IgE. In contrast, DNCB and OXA failed to elicit the pathologic changes in the nasal airways and cytokine changes in the lung. This model may be useful for identifying other chemical respiratory allergens.


http://toxsci.oupjournals.org/cgi/content/abstract/kfi155v1
Studies have shown using immunohistochemical staining that the MT-1 and MT-2 proteins (MT-1/2) are overexpressed in a substantial sub-set of ductal breast cancers, that overexpression occurs early in the disease process, and is indicative of a poor prognosis. Normal ductal breast epithelium fails to immunostain for the MT-1/2 protein where as the myoepithelial cells of the ducts stain intensely. There is no information regarding the expression of the mRNAs for the 8 active MT-1 and MT-2 genes in normal breast duct epithelium. Microdissection of normal breast samples was used to obtain total RNA from enriched populations of ductal epithelium and myoepithelium. Analysis by RT-PCR demonstrated that the identity of the MT isoform-specific genes expressed (MT-2A and MT-1X) and their relative levels of expression were similar between the myoepithelial and ductal components. These findings indicate that the ductal and myoepithelial components express similar amounts of MT-2A and MT-1X mRNAs, but have distinctly different expression of the MT-1/2 protein. Confluent cultures of MCF-10A breast epithelial cells were exposed to Cd+2 to test for evidence of post-transcriptional regulation of MT-1/2 protein accumulation in ductal epithelium. It was demonstrated that Cd+2 elicited only a marginal induction of MT-1E, MT-1X or MT-2A mRNAs; where as, there was a marked increase in MT-1/2 protein, reaching levels of 6% of total cell protein under conditions of extended exposure. This study suggests that the mechanism underlying the finding of increased MT-1/2 protein expression in ductal breast cancer may involve in part the post-transcriptional regulation of MT-1/2 protein expression.


http://toxsci.oupjournals.org/cgi/content/abstract/67/2/219

The application of gene expression profiling technology to examine multiple genes and signaling pathways simultaneously promises a significant advance in understanding toxic mechanisms to ultimately aid in protection of public health. Public and private efforts in the new field of toxicogenomics are focused on populating databases with gene expression profiles of compounds where toxicological and pathological endpoints are well characterized. The validity and utility of a toxicogenomics is dependent on whether gene expression profiles that correspond to different chemicals can be distinguished. The principal hypothesis underlying a toxicogenomic or pharmacogenomic strategy is that chemical-specific patterns of altered gene expression will be revealed using high-density microarray analysis of tissues from exposed organisms. Analyses of these patterns should allow classification of toxicants and provide important mechanistic insights. This report provides a verification of this hypothesis. Patterns of gene expression corresponding to liver tissue derived from chemically exposed rats revealed similarity in gene expression profiles between animals treated with different agents from a common class of compounds, peroxisome proliferators [clofibrate (ethyl-p-chlorophenoxyisobutyrate), Wyeth 14,643 ([4-chloro-6(2,3-xylidino)-2-pyrimidinylthio]acetic acid), and gemfibrozil (5-2[2,5-dimethylphenoxy]2-2-dimethylpentanoic acid]), but a very distinct gene expression profile was produced using a compound from another class, enzyme inducers (phenobarbital).


http://toxsci.oupjournals.org/cgi/content/abstract/81/1/78

The potential for a variety of environmental contaminants to disturb endocrine function in wildlife and humans has been of recent concern. While much effort is being focused on the assessment of effects mediated through steroid hormone receptor-based mechanisms, there are potentially
several other mechanisms that could lead to endocrine disruption. Recent studies have demonstrated that a variety of xenobiotics can alter the gene expression or activity of enzymes involved in steroidogenesis. By altering the production or catalytic activity of steroidogenic or steroid-catabolizing enzymes, these chemicals have the potential to alter the steroid balance in organisms. To assess the potential of chemicals to alter steroidogenesis, an assay system was developed using a human adrenocortical carcinoma cell line, the H295R cell line, which retains the ability to synthesize most of the important steroidogenic enzymes. Methods were developed, optimized, and validated to measure the expression of 10 genes involved in steroidogenesis by the use of real-time quantitative reverse transcriptase PCR. The effects of several model chemicals known to alter steroid metabolism, both inducers and inhibitors, were assessed. Similar expression patterns were observed for chemicals acting through common mechanisms of action. Time-course studies demonstrated distinct time-dependent expression profiles for chemicals able to modulate steroid metabolism. The assay, which allows simultaneous analysis of the expression of numerous steroidogenic enzymes, would be useful as a sensitive and integrative screen for the many effects of chemicals on steroidogenesis.


Aluminum maltolate (Al-malt) causes neurodegeneration following in vivo exposure, and apoptosis plays a prominent role. The objective of this study was to define the form of cell death induced by Al-malt and to establish an in vitro model system amenable to mechanistic investigations of Al-malt-induced cell death. Neuro-2a cells, a murine neuroblastoma cell line, were treated with Al-malt for 24 h, following which mode of cell death and alterations in apoptosis-related gene expression were studied. Al-malt concentration-dependently increased cell death. The mode of cell death was a combination of apoptosis and necrosis. Treatment with Al-malt resulted in caspase 3 activation and the externalization of phosphatidyl serine, both indicative of apoptosis. In addition, nuclear condensation and fragmentation were evident. Interestingly, pretreatment with cycloheximide (CHX), a potent protein synthesis inhibitor markedly reduced Al-malt-induced apoptosis, indicating that altered gene expression was critical for this form of cell death. Pretreatment with CHX had no effect on necrosis induced by Al-malt. Analysis of gene expression showed that p53 mRNA was increased following treatment with Al-malt. This increase was accompanied by a marked inhibition of Bcl2 expression and an increase in BAX expression, a pattern of gene expression suggestive of a pro-apoptotic shift. Results show for the first time that p53 is induced by Al in neuron-like cells and suggest that the p53-dependent intrinsic pathway may be responsible for Al-induced apoptosis. Future studies investigating the role of p53 in Al neurotoxicity both in vivo and in vitro are warranted.


Effects of a commercial polychlorinated biphenyls mixture, Kanechlor-500 (KC500), on the levels of serum thyroid hormones such as total thyroxine (T4) and triiodothyronine (T3) were examined comparatively in male Wistar rats and ddy mice. Serum T4 levels were significantly decreased in both rats and mice 4 days after a single ip injection of KC500 (100 mg/kg body weight), whereas decreased levels of T3 were observed in mice but not in rats. In addition, no significant change in the level of serum thyroid stimulating hormone was observed in either rats or mice. Hepatic UDP-glucuronosyltransferases (UDP-GTs) UGT1A1 and UGT1A6, which efficiently mediate
glucuronidation of T4 and promote the excretion of the hormones, were induced by KC500 in rats but not in mice. Hepatic microsomal cytochrome P450 (P450) content and the microsomal activity for 7-ethoxy-, 7-pentoxy-, and 7-benzoxyloxy-resorufin dealkylations were significantly increased by KC500 in both rats and mice, although the magnitude of increase in the enzyme activities was higher in rats than in mice. The difference in the increase in the activity of microsomal enzymes, including UDP-GT and P450, between KC500-treated rats and mice was not correlated with that in the level of hepatic methylsulfonyl-PCB metabolites. In the present study, we found for the first time that the decrease in serum T4 levels by KC-500 in mice occurred without increase in hepatic UDP-GTs, UGT1A1 and UGT1A6, responsible for T4 glucuronidation. The present findings further suggested that although the decrease in serum T4 levels in KC500-treated rats would occur at least in part through the induction of the UDP-GTs, it might not be dependent on only the increase in the enzymes.


http://toxsci.oupjournals.org/cgi/content/abstract/73/2/348

Uncouplers of oxidative phosphorylation have relevance to bioenergetics and obesity. The mechanisms of action of chemical uncouplers of oxidative phosphorylation on biological systems were evaluated using differential gene expression. The transcriptional response in human rhabdomyosarcoma cell line (RD), was elucidated following treatment with carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), a classical uncoupling agent. Changes in mitochondrial membrane potential were used as the biological dosimeter. There was an increase in membrane depolarization with increasing concentrations of FCCP. The concentration at 75% uncoupling (20 μM) was chosen to study gene expression changes, using cDNA-based large-scale differential gene expression (LSDGE) platforms. At the above concentration, subtle light microscopic and clear gene expression changes were observed at 1, 2, and 10 h. Statistically significant transcriptional changes were largely associated with protein synthesis, cell cycle regulation, cytoskeletal proteins, energy metabolism, apoptosis, and inflammatory mediators. Bromodeoxyuridine (BrdU) and propidium iodide (PI) assays revealed cell cycle arrest to occur in the G1 and S phases. There was a significant initial decrease in the intracellular adenosine triphosphate (ATP) concentrations. The following seven genes were selected as potential molecular markers for chemical uncouplers: seryl-tRNA synthetase (Ser-tRS), glutamine-hydrolyzing asparagine synthetase (Glut-HAS), mitochondrial bifunctional methylenetetrahydrofolate dehydrogenase (Mit BMD), mitochondrial heat shock 10-kDa protein (Mit HSP 10), proliferating cyclic nuclear antigen (PCNA), cytoplasmic beta-actin (Act B), and growth arrest and DNA damage-inducible protein 153 (GADD153). Transcriptional changes of all seven genes were later confirmed with reverse transcription-polymerase chain reaction (RT-PCR). These results suggest that gene expression changes may provide a sensitive indicator of uncoupling in response to chemical exposure.


http://toxsci.oupjournals.org/cgi/content/abstract/72/2/339

Exposure to particulate matter (PM) may exacerbate preexisting respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis, and pneumonia. However, few experimental studies have addressed the effects of PM on lower respiratory tract (LRT) viral
infection. Respiratory syncytial virus (RSV) is a major etiological agent for LRT infections in infants, the elderly, and the immunocompromised and may lead to chronic wheezing and the development of asthma in children. In this study, we examined the effects of carbon black (CB) on RSV-induced pulmonary inflammation, chemokine and cytokine expression, and airway hyperresponsiveness in a mouse model of RSV. Female BALB/c mice were instilled via the trachea (i.t.) with 1 x 10^6 plaque forming units (pfu) RSV or with uninfected culture media. On day 3 of infection, mice were i.t. instilled with either 40 \( \mu \)g ultrafine CB particles or with saline. End points were examined on days 4, 5, 7, and 14 of RSV infection. Viral titer and clearance in the lung were unaffected by CB exposure. Neutrophil numbers were elevated on days 4 and 7, and lymphocyte numbers were higher on days 4 and 14 of infection in CB-exposed, RSV-infected mice. CB exposure also enhanced RSV-induced airway hyperresponsiveness to methacholine, bronchoalveolar lavage (BAL) total protein, and virus-associated chemokines monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1{alpha}), and regulated upon activation, normal T cell expressed and secreted (RANTES). MIP-1{alpha} mRNA expression was increased in the alveolar epithelium, where ultrafine particles deposit in the lung. These data demonstrate a synergistic effect of ultrafine CB particles on RSV infection, and suggest a potential mechanism for increased respiratory infections in human populations after PM exposure.

http://toxsci.oupjournals.org/cgi/content/abstract/68/2/465

2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental toxin, has been shown to cause a human skin pathology called chloracne. The majority of laboratory mouse strains, with the exception of mice bearing a mutation in the hairless gene, fail to display overt signs of chloracne upon exposure to TCDD. As a result, only minimal data exist on the effects of TCDD in adult haired mice and no data exist on the effects of TCDD in developing mouse skin. Here we report that TCDD affects the temporal expression of protein markers of keratinocyte terminal differentiation during murine skin morphogenesis. Immunohistochemical analysis of E16 mice reveals accelerated expression of the intermediate filament-associated protein filaggrin in response to TCDD. At a later developmental time and after birth, expression of filaggrin and loricrin is indistinguishable between treatment and control groups. At E16 expression of keratins 5, 6, and 10 are unaltered in TCDD-exposed individuals and TUNEL analysis shows no apoptotic cells in the basal and spinous layers of either treatment or control groups. At E16, immunohistochemical analysis of AhR-null mouse skin reveals accelerated filaggrin expression in both vehicle and TCDD exposed animals. We therefore hypothesize that AhR acts as a modulator of late stage keratinocyte terminal differentiation.

http://toxsci.oupjournals.org/cgi/content/abstract/68/1/93

Although they are known to be effective antidiabetic agents, little is published about the toxic effects of carnitine palmitoyltransferase-1 (CPT-1) inhibitors, such as etomoxir (ET). These compounds inhibit mitochondrial fatty acid \( \beta \)-oxidation by irreversibly binding to CPT-1 and preventing entry of long chain fatty acids into the mitochondrial matrix. Treatment of HepG2 cells with 1 mM etomoxir for 6 h caused significant modulations in the expression of several redox-related and cell cycle mRNAs as measured by microarray analysis. Upregulated mRNAs included heme oxygenase 1 (HO1), 8-oxoguanine DNA glycosylase 1 (OGG1), glutathione reductase
(GSR), cyclin-dependent kinase inhibitor 1A (CDKN1 [p21waf1]) and Mn+ superoxide dismutase precursor (SOD2); while cytochrome P450 1A1 (CYP1A1) and heat shock 70kD protein 1 (HSPA1A) were downregulated. Real time quantitative PCR (RT-PCR) confirmed the significant changes in 4 of 4 mRNAs assayed (CYP1A1, HO1, GSR, CDKN1), and identified 3 additional mRNA changes; 2 redox-related genes, {gamma}-glutamate-cysteine ligase modifier subunit (GCLM) and thioredoxin reductase (TXNRD1) and 1 DNA replication gene, topoisomerase II(alpha) (TOP2A). Temporal changes in selected mRNA levels were examined by RT-PCR over 11 time points from 15 min to 24 h postdosing. CYP1A1 exhibited a 38-fold decrease by 4 h, which rebounded to a 39-fold increase by 20 h. GCLM and TXNRD1 exhibited 13- and 9-fold increases, respectively at 24 h. Etomoxir-induced oxidative stress and impaired mitochondrial energy metabolism were confirmed by a significant decrease in reduced glutathione (GSH), reduced/oxidized glutathione ratio (GSH/GSSG), mitochondrial membrane potential (MMP), and ATP levels, and by concurrent increase in oxidized glutathione (GSSG) and superoxide generation. This is the first report of oxidative stress caused by etomoxir.


http://toxsci.oupjournals.org/cgi/content/abstract/82/1/318

Hydrazine (HD) and acetylhydrazine (AcHD) are metabolites of the antituberculosis drug isoniazid (INH) that have been implicated in INH-induced liver damage. The hepatotoxicity of AcHD and HD were compared in adult male C57Bl/6J mice by evaluating hepatic histopathology, plasma biochemistry, and hepatic gene expression. By all measures, HD had significantly greater effects than AcHD. There was no evidence of liver damage following exposure to AcHD (300 mg/kg, po). However, HD at this dose caused marked hepatic necrosis, macrovesicular degeneration, and steatosis. Lipid accumulation was initiated 2 h after HD exposure, with hepatic macrovesicular degeneration evident after 4 h, and severe necrosis by 36 h. Gene expression profiles were compared 24 h following 100 mg/kg po of HD or AcHD. HD changed the hepatic expression of more genes than AcHD, particularly lipid synthesis, transport, and metabolism genes that may be involved in steatosis. Hepatic expression of genes regulated by peroxisome proliferator activated receptors (PPAR) and sterol regulatory element binding protein (SREBP) transcription factors was increased only by HD. The hepatotoxicity and hepatic gene expression profile of HD, but not AcHD, indicate that exposure to HD initiates a process whereby the production and intracellular transport of hepatic lipids is favored over the removal of fatty acids and their metabolites.


http://toxsci.oupjournals.org/cgi/content/abstract/82/2/534

Liver slice viability is extended to 96 h for rat, expanding the use of this in vitro model for studying mechanisms of injury and repair, including pathways of fibrosis. The contributing factors to increased organ slice survival consist of the use of a preservation solution for liver perfusion and slice preparation, obtaining rats that are within the weight range of 250-325 g, placing a cellulose filter atop the titanium mesh roller-insert to support the slice, and maintaining the slices in an optimized culture medium which is replaced daily. The liver slices remain metabolically active, synthesizing adenosine triphosphate (ATP), glutathione, and glycogen, and exhibit preserved organelle integrity and slice morphology. Slice preparation results in 2-cut surfaces which likely triggers a repair and regenerative response. The fibrogenic pathways are evident by the activation of stellate cells, the proliferation of myofibroblast-like cells, and an increased collagen deposition by 48 h. Markers indicative of activated stellate cells, {alpha-smooth muscle actin,
collagen 1α1, desmin, and HSP47 are substantiated by real time-PCR. Increased staining of α-smooth muscle actin initially around the vessels and by 72-96 h in the tissue is accompanied by increased collagen staining. Microarray gene expression revealed extracellular matrix changes with the up-regulation of cytoskeleton, filaments, collagens, and actin genes; and the down-regulation of genes linked with lipid metabolism. The improvements in extending liver slice survival, in conjunction with its three-dimensional multi-cellular complexity, increases the application of this in vitro model for investigating pathways of injury and repair, and fibrosis.


http://toxsci.oupjournals.org/cgi/content/abstract/76/1/151

Adult zebrafish completely regenerate their caudal fins following partial amputation. Fin regrowth can easily be monitored in vivo and regenerating tissues can be used to study this dynamic developmental process. In this study we determined that fin regeneration is significantly affected by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Zebrafish caudal fins were partially amputated, and the fish received intraperitoneal (ip) injection of 2.8, 14, or 70 ng/g weight TCDD or vehicle control. By 7 days postamputation, fish exposed to the highest concentration of TCDD regenerated 15% of their fin compared to 65% regrowth in control fish. To determine if this effect was stage specific, zebrafish were exposed to 70 ng/g TCDD on 1, 2, 3, or 4 days postamputation. Fin regeneration was significantly inhibited at all time points following TCDD exposure. TCDD exposure also induced hyperpigmentation in de novo tissue. Zebrafish were dosed with BrdU, following fin amputation and TCDD exposure, to study changes in cell proliferation. By 4 days postamputation, cell proliferation rates were significantly lower in TCDD-exposed fish. TCDD toxicity is mediated through the aryl hydrocarbon receptor (AHR), and RT-PCR experiments confirmed AHR2, ARNT2b, and TCDD-dependent CYP1A expression in the regenerating tissue. These results demonstrate that zebrafish caudal fin regeneration is a unique model to investigate molecular mechanism(s) of TCDD toxicity.


Increased iron store in the body may increase the risk of many diseases such as cancer and inflammation. However, the precise pathogenic mechanism of iron has not yet been elucidated. In the present study, the early biological responses of cells to iron treatment were investigated in AP-1 luciferase reporter stably transfected mouse epidermal JB6 cells and primary rat hepatocytes. It was shown that water-soluble iron compounds, such as FeSO4 and Fe2(SO4)3, were more active in inducing AP-1 in JB6 cells than water-insoluble iron compounds, such as Fe2O3 and FeS. Iron stimulated mitogen-activated protein kinase (MAPK) family members of extracellular signal-regulated kinases (ERKs) and p38 MAPK but not c-jun NH2 terminal kinases.
(JNKs), both in JB6 cells and in primary rat hepatocytes, as determined by the phosphorylation assay. Interestingly, the increase in AP-1 luciferase activity by iron was inhibited by the pretreatment of the cells with PD98059, a specific MEK1 inhibitor, and SB202190, a p38 kinase inhibitor. Levels of interleukin-6 (IL-6), a pro-inflammatory cytokine, were increased in JB6 cells by iron in a dose-dependent manner. The increase in IL-6 and its mRNA by iron was also eliminated by the pretreatment of the cells with PD98059 and SB202190. Since the IL-6 promoter contains an AP-1 binding site, our studies indicate that the iron-induced IL-6 gene expression may be mediated through ERKs and p38 MAPK pathways, possibly one of the important mechanisms for the pathogenesis of iron overload.


http://www.sciencedirect.com/science/article/B6TCN-44N3BXJ-7/2/61cb99fe146d9e6b6895c57f0a3efa41

The mechanism of cadmium-mediated hepatotoxicity has been the subject of numerous investigations, principally in hepatocytes. Although, some uncertainties persist, sufficient evidence has emerged to provide a reasonable account of the toxic process in parenchymal cells. However, there is no information about the effect of cadmium in other hepatic cell types, such as stellate cells (fat storing cells, Ito cells, perisinusoidal cells, parasinusoidal cells, lipocytes). Hepatic stellate cells (HSC) express a quiescent phenotype in a healthy liver and acquire an activated phenotype in liver injury. These cells play an important role in the fibrogenic process. The objective of this study was to investigate the effect of a 24 h treatment of low Cd concentrations in glutathione content, lipid peroxidation damage, cytosolic free Ca, antioxidant enzyme activities: glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase along with the capacity of this heavy metal to induce metallothionein II and [alpha]1collagen (I) in an hepatic stellate cell line (CFSC-2G). Cd-treated cells increased lipid peroxidation and the content of cytosolic free calcium, decreased glutathione content and superoxide dismutase, glutathione peroxidase and catalase activity. Cd was able to induce the expression of the metallothionein II and [alpha]1collagen (I) gene, that was not described in this cell type. Cadmium may act as a pro-fibrogenic agent in the liver probably by inducing oxidative damage by enhancing lipid peroxidation and altering the antioxidant system of the cells. Although, the exact role metallothionein induction plays in this process is unknown, it probably, provides a cytosolic pool of potential binding sites to sequester ionic Cd, thereby decreasing its toxicity.


Formaldehyde (FA), an occupational and environmental toxicant used extensively in the manufacturing of many household and personal use products, is known to induce squamous cell carcinomas in the nasal turbinates of rats and mice and squamous metaplasia in monkey noses. Tissue responses to FA include a dose dependent epithelial degeneration, respiratory cell hypertrophy, and squamous metaplasia. The primary target for FA-induced toxicity in both rodents and monkeys is the respiratory nasal epithelium. FA increases nasal epithelial cell proliferation and DNA-protein crosslinks (DPX) that are associated with subsequent nasal cancer development. To address the acute effects of FA exposure that might contribute to known pathological changes, cDNA gene expression analysis was used. Two groups of male F344 rats received either 40 ul of distilled water or FA (400 mM) instilled into each nostril. Twenty-four
hours following treatment, nasal epithelium was recovered from which total RNA was used to generate cDNA probes. Significance analysis of microarrays (SAM) hybridization data using Clontech(TM) Rat Atlas 1.2 arrays revealed that 24 of the 1185 genes queried were significantly up-regulated and 22 genes were significantly downregulated. Results for ten of the differentially expressed genes were confirmed by quantitative real time RT PCR. The identified genes with FA-induced change in expression belong to the functional gene categories xenobiotic metabolism, cell cycle, apoptosis, and DNA repair. These data suggest that multiple pathways are dysregulated by FA exposure, including those involved in DNA synthesis/repair and regulation of cell proliferation. Differential gene expression profiles may provide clues that could be used to define mechanisms involved in FA-induced nasal cancer.


Recent research suggests that high-altitude hypoxia may serve as a model for prolonged oxidative stress in healthy humans. In this study, we investigated the consequences of prolonged high-altitude hypoxia on the basal level of oxidative damage to nuclear DNA in muscle cells, a major oxygen-consuming tissue. Muscle biopsies from seven healthy humans were obtained at sea level and after 2 and 8 weeks of hypoxia at 4100 m.a.s.l. We found increased levels of strand breaks and endonuclease III-sensitive sites after 2 weeks of hypoxia, whereas oxidative DNA damage detected by formamidopyrimidine DNA glycosylase (FPG) protein was unaltered. The expression of 8-oxoguanine DNA glycosylase 1 (OGG1), determined by quantitative RT-PCR of mRNA levels did not significantly change during high-altitude hypoxia, although the data could not exclude a minor upregulation. The expression of heme oxygenase-1 (HO-1) was unaltered by prolonged hypoxia, in accordance with the notion that HO-1 is an acute stress response protein. In conclusion, our data indicate high-altitude hypoxia may serve as a good model for oxidative stress and that antioxidant genes are not upregulated in muscle tissue by prolonged hypoxia despite increased generation of oxidative DNA damage.


The UPL rat is a newly developed hereditary cataract model. We previously found that Ca2+ concentrations in UPL rat lenses increase with the development of cataract, and that the administration of disulfiram and aminoguanidine ameliorates the increase in Ca2+ and the development of cataract in UPL rats. In this study, we determined the expression and activity of plasma membrane Ca2+-ATPase (PMCA) in lenses of normal and UPL rats. We also determined the ATP content in UPL rat lenses and the effects of disulfiram and aminoguanidine administration. Expression of PMCA mRNA in UPL rat lenses, determined by a reverse transcription-PCR method, increased during the development of cataract. Ca2+-ATPase activity in UPL rat lenses also increased with the progression of lens opacification. On the other hand, ATP decreased markedly in UPL rat lenses, and the administration of disulfiram and aminoguanidine attenuate the ATP decrease. These results suggest that an ATP decrease cause cataract development and an increased Ca2+ may upregulate PMCA expression in UPL rat lenses. Disulfiram and aminoguanidine attenuate the decrease in ATP, resulting in a delay in cataract development.

http://www.sciencedirect.com/science/article/B6TCN-448BG0N-6/2/02862c8f8d95353a92b75ba46a97be4e

CYP2A6 (cytochrome P450 2A6), which was first identified as the human coumarin 7-hydroxylase, is the most important enzyme in nicotine C-oxidation. The enzyme also metabolically activates the tobacco specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) in vitro. Polymorphisms in the CYP2A6 gene may thus impact on both smoking behavior and lung cancer susceptibility. Several different genotyping methods have been reported with conflicting results in the frequencies of CYP2A6 polymorphic variants. Thus we decided to perform a sequence analysis of the entire CYP2A6 gene. Sequencing confirmed the published CYP2A6 cDNA sequence. However, intron sequences differed considerably from the reported sequence of the CYP2A6*3 (v2) variant. Our analyses revealed that parts of introns shared homologies with the published sequence of CYP2A13. Based on our sequence data we developed a one step protocol for specific amplification of exon 3 of CYP2A6. The resulting PCR product can be used directly for restriction endonuclease digestion with XcmI and DdeI to determine the frequencies of the reported variant alleles CYP2A6*2 and CYP2A6*3. In a population of 305 African-Americans and 145 Caucasians, we found allele frequencies of 0.003 (2/610) for CYP2A6*2 and 0 (0/610) for CYP2A6*3 in African-Americans and allele frequencies of 0.014 (4/290) and 0 (0/290) in Caucasians. We conclude that both alleles are considerably less frequent in populations than previously reported.


http://www.sciencedirect.com/science/article/B6TCN-4CHS17S-1/2/3a68eb8e91c766a6ab812c701b353e87

Inhalation of fungal spores may cause inflammation and respiratory diseases, such as bronchitis, allergic alveolitis, and asthma. Alveolar macrophages provide the first line of defense in the respiratory tract. To examine the cellular mechanisms involved in Aspergillus fumigatus-induced airway inflammation, mouse macrophage cell line (RAW 264.7) cells were exposed for 2 h or 6 h to graded doses of A. fumigatus spores that were either alive or heat-killed. Furthermore, the ability of the cells to phagocytose the spores was visualized by electron microscopy. Expression of selected cytokines and chemokines was assessed by a real time quantitative PCR method and by enzyme-linked immunoabsorbent assay (ELISA) after exposure. A significant increase in mRNA expression of TNF-[alpha], MIP-1[alpha], MIP-1[beta], and MCP-1 was observed with a maximal induction at 6 h after exposure to the highest (1 x 10^7) concentration of live spores. Similar response was not detected with heat-killed spores in the expression of chemokines and cytokines, even though there were no differences between the phagocytosis of live and heat-killed spores. These results suggest that exposure to live spores of A. fumigatus can modulate the expression of proinflammatory cytokines and chemokines in mouse macrophages and thus influence the development of inflammatory processes in the airways.

Long-term cadmium exposure leads to mitochondrial dysfunction in the proximal tubular epithelial cells. Mitochondrial DNA deletion may contribute to the pathogenesis of cadmium-induced nephropathy. The aim of our study is to clarify the accumulation of mitochondrial DNA deletion and mitochondrial dysfunction in the renal cortex of rats injected three times/week with 1 ml of 1 mM CdCl₂ or saline for 80 weeks. After 40-week cadmium injection, mitochondrial number diminished, and cadmium in the renal cortex reached a saturation level. At this time interval, nearly 30% of cadmium in the whole cell fraction was found in the mitochondria. Cytochrome c oxidase (COX) activity in the proximal tubular epithelial cells decreased after 40-week exposure of cadmium. Oxidized phosphatidylcholine (oxPC) started to accumulate in the cytochrome c-positive mitochondria in some tubular epithelial cells after 80-week exposure. After 40 weeks, accumulation of the 4834-bp deletion in mitochondrial DNA was evident in both control and cadmium-treated groups. However, the amount of accumulated mitochondrial DNA deletion tended to increase after 40-week exposure, and was significantly greater after 80 weeks of exposure, compared to the control. Our results indicate that long-term cadmium exposure in rats accelerates accumulation of 4834-bp mitochondrial DNA deletions and impairment of mitochondrial function associated with accumulation of oxidized product.


Styrene is widely used in the production of various plastics, synthetic rubber and resins. The aim of this study was to evaluate if individual polymorphisms in xenobiotic metabolizing enzymes, related with the metabolic fate of styrene, could modify individual susceptibility to the possible genotoxic effects of the styrene exposure. Twenty-eight reinforced plastic workers and 28 control subjects were studied. In the selected population the urinary styrene metabolites mandelic (MA) and phenylglyoxylic (PGA) acids were quantified, sister chromatid exchanges (SCE) and micronuclei (MN) were assessed in peripheral lymphocytes and all the subjects were genotyped for GSTM1, GSTT1 (gene deletions), GSTP1 (codon 105 ile => val), EPHX1 (codons 113 tyr => his and 139 his => arg) and CYP2E1 (Dral polymorphism in intron 6). The results obtained showed a significant difference between the levels of SCE, but not in MN levels, in exposed workers as compared with the control group. The GSTP1 and CYP2E1 individual genotypes modulate the baseline levels of SCE that are lower in non-wild type individuals for both polymorphisms. The GSTM1 null individuals with low levels of exposure have significantly higher urinary levels of MA+PGA. The present data seem to suggest that apart from the methodology usually used for monitoring populations occupationally exposed to styrene (urinary metabolites and biomarkers of early biological effects) the analysis of individual genotypes associated with the metabolic fate of styrene should also be carried out in order to evaluate the individual genetic susceptibility of exposed populations.

Diisononyl phthalate (DINP) is a compound widely used as a plasticizer in the production of polyvinyl chloride products. Chronic exposure to DINP leads to liver cancer in rats and mice. Many phthalates are considered to be relatively weak peroxisome proliferators (PP), a group of rodent hepatocarcinogens that cause a variety of adaptive responses in liver through the PP-activated receptor alpha (PPAR[alpha]). The objectives of this study were to determine whether DINP-induced effects in the liver associated with carcinogenesis are mediated by PPAR[alpha] and to identify novel gene targets of DINP. Male and female SV129 wild-type, SV129 PPAR[alpha]-null, and B6C3F1 mice were administered DINP by gavage or in the feed. Transcript profile technology and reverse transcriptase (RT)-polymerase chain reaction (PCR) were used to identify gene targets. Dose-dependent increases in relative liver weights were dependent on PPAR[alpha] in 10- or 12-week-old male and female mice and 30-week-old male mice. Female 30-week-old mice exhibited PPAR[alpha]-independent increases in relative liver weights. Increases in hepatocyte proliferation, palmitoyl-CoA oxidase (PCO) activity, and levels of enzymes involved in [beta]- and [omega]-oxidation of fatty acids were shown to be dependent on PPAR[alpha]. Five novel genes were shown to be altered in the livers of female wild-type mice after a 3-week exposure, but not in PPAR[alpha]-null, mice. These genes included those involved in DNA repair and recombination (ATP-dependent helicase and Endonuclease III homolog), drug metabolism (Cyp2a4) and protein trafficking (FKBP-1, FKBP-13). An additional gene (Cyp2d9) was shown to be down-regulated in wild-type mice but up-regulated in PPAR[alpha]-null mice indicating more complex regulation by PPAR[alpha] and additional factors. These data support the hypothesis that PPAR[alpha] plays a dominant role in mediating the effects associated with hepatocarcinogenesis after DINP exposure.


Assessment of allergenic potency of low molecular weight compounds is generally performed using animal models, such as the guinea pig maximisation test and the murine local lymph node assay (LLNA). Progress in unravelling the mechanisms of skin sensitisation, including effects on the production of cytokines by the different cell types of the skin, provides us with the opportunity to develop in vitro tests as an alternative to in vivo sensitisation testing. The aim of the present study was to establish an in vitro method to assess the potency of allergens, on the basis of their induction of cytokine production by murine and human keratinocytes. In the present study we used test systems comprised of the murine epidermal keratinocyte cell line HEL-30 and the human keratinocyte cell line HaCaT. We exposed these cell lines to the allergens ethyl-p-aminobenzoate (benzocaine), diethylamine (DEA), 2,4-dinitrochlorobenzene (DNCB), and phthalic anhydride (PA). IL-1[alpha] and IL-18 dose-response data were evaluated by non-linear regression analysis and at a stimulation index of 3 of cytokine production of treatment versus control, the corresponding allergen concentration was calculated. For HEL-30, for both cytokines DNBC showed the strongest potency followed in this order by PA, benzocaine, and DEA. This classification was similar to our previous findings obtained in the LLNA. For HaCaT, unfortunately, such ranking proved to be much less feasible. In conclusion, to assess the potency of allergens the murine keratinocyte cell line HEL-30 may be a useful in vitro test system, alternative to in vivo models, although this requires further testing using a much wider range of compounds.

The local lymph node assay (LLNA) is used to identify allergens by means of dermal exposure. For hazard identification, besides identification also the distinction between contact and respiratory allergens is of importance. We have previously shown that a modified LLNA can be used to identify respiratory allergens, on the basis of Con A induced IL-4 production. Here we show a good qualitative correlation between mRNA expression and production of IFN-[gamma] and IL-4. This suggests that distinction between contact and respiratory allergens may also be studied at the mRNA expression level. Secondly, another assay, similar to the modified LLNA but differing in the duration and the number of allergen applications as well as in the ex vivo culture conditions, here denoted as 'longer' assay, has been reported to be able to identify contact allergens, on the basis of (spontaneous) IFN-[gamma] production. In the present study we have compared these assays. Similar to our previous findings, in the modified LLNA exposure to the respiratory allergen trimellitic anhydride (TMA) resulted in a ~10-fold higher Con A induced IL-4 production compared with the contact allergen dinitrochlorobenzene (DNCB), while exposure to both allergens resulted in a similar Con A induced IFN-[gamma] production. In the 'longer' assay, TMA exposure resulted in Con A induced IL-4 production whereas DNCB exposure did not. Importantly, only a 2-fold higher spontaneous IFN-[gamma] production was induced by DNCB compared with TMA, the difference being not statistically significant. Thus, although the 'longer' assay indeed showed a somewhat higher IFN-[gamma] induction by DNCB compared with TMA, the magnitude and robustness of this effect question its applicability. These results favor the modified LLNA since it is shorter, and combines identification of allergens (by cell proliferation) with identification of respiratory allergens (by IL-4 production). Compounds that induce cell proliferation with a low concomitant IL-4 production may thus be identified as contact allergens, although the need to positively identify such allergens remain.


Rat thymocytes and splenocytes were exposed in vitro to the model compounds Cyclosporin A (CsA), an immunosuppressive drug, and bis(tri-n-butyltin)oxide (TBTO), an immunotoxic environmental contaminant. The lymphocyte transformation test (LTT), cytokine (receptor) mRNA expression (RT-PCR and dot blot hybridisation), and flow cytometry were evaluated as assays for in vitro immunotoxicity, at dose levels that did not show effects on viability, this being the aim of the study. LTT and RT-PCR proved useful assays. Lymphocyte transformation was suppressed by both compounds, while IL-2 mRNA expression was suppressed by CsA but not by TBTO, and both compounds suppressed IL-2R mRNA expression in splenocytes but not in thymocytes. Furthermore, the data obtained suggest that antiproliferative effects may be more relevant than apoptosis induction for TBTO induced thymus atrophy.

http://www.sciencedirect.com/science/article/B6WXH-4C0V5S4-1/2/63ea8338a7b897b93357b23949f74a86

Cisplatin is a widely used anticancer drug, but at high dose, it can produce undesirable side effects such as hepatotoxicity. Because Curcuma xanthorrhiza Roxb. (Zingiberaceae) has been traditionally used to treat liver disorders, the protective effect of xanthorrhizol, which is isolated from C. xanthorrhiza, on cisplatin-induced hepatotoxicity was evaluated in mice. The pretreatment of xanthorrhizol (200 mg/kg/day, po) for 4 days prevented the hepatotoxicity induced by cisplatin (45 mg/kg, ip) with statistical significance. Interestingly, it abrogated cisplatin-induced DNA-binding activity of nuclear factor-kappaB (NF-[kappa]B), which consequently affected mRNA expression levels of NF-[kappa]B-dependent genes, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), even in part. It also attenuated the cisplatin-suppressed DNA-binding activity of activator protein 1 (AP-1). Using differential display reverse transcription-polymerase chain reaction (DDRT-PCR), seven upregulated genes including S100 calcium binding protein A9 (S100A9) mRNA and antigenic determinant for rec-A protein mRNA and five downregulated genes including caseinolytic protease X (ClpX) mRNA and ceruloplasmin (CP) mRNA by cisplatin were identified. Although these mRNA expression patterns were not totally consistent with gel shift patterns, altered expression levels by cisplatin were reversed by the pretreatment of xanthorrhizol. In conclusion, the ability of xanthorrhizol to regulate the DNA-binding activities of transcription factors, NF-[kappa]B and AP-1, could be one possible mechanism to elucidate the preventive effect of xanthorrhizol on cisplatin-induced hepatotoxicity. Furthermore, genes identified in this study could be helpful to understand the mechanism of cisplatin-induced hepatotoxicity. Finally, the combination treatment of xanthorrhizol and cisplatin may provide more advantage than single treatment of cisplatin in cancer therapy.


http://www.sciencedirect.com/science/article/B6WXH-47RYSNF-4/2/e14863329ee59ff7df018f4c3cd54ff1

BALB/c mice were sensitized to ovalbumin by systemic injection and then exposed for up to 8 weeks to ovalbumin aerosols in whole body chambers. A pattern of airway inflammation, mucous cell hypertrophy and hyperplasia, and airway remodeling with submucosal fibrosis was observed as lesions evolved over time. Larger conducting airways were removed from the lungs by microdissection. Airway fibrosis was quantified by direct assay for collagen content, which was significantly increased after 4 and 8 weeks of exposure to ovalbumin aerosol. Based upon PCR analysis of mRNA levels in the airways, most of the newly synthesized collagen was Type I. Relaxin, administered by continuous infusion over the second half of a 4-week exposure to ovalbumin, was able to inhibit the accumulation of collagen in the airways of exposed mice. Thus, stimulation of collagen degradation by an activator of collagen breakdown by matrix metalloproteinases appears to be an effective therapeutic strategy in prevention of airway fibrosis in this animal model. Whole body plethysmography of unrestrained mice indicated functional changes in airway reactivity in the lungs of exposed animals occurring in conjunction with the reported structural changes. This result indicates that the ovalbumin-exposed mouse may be a suitable model for examining structure-function relationships in the lungs of animals with a predictable time course of airway inflammation, remodeling, and fibrosis and for testing potential new drugs for treatment of asthma or chronic bronchitis at a mechanistic level.

http://www.sciencedirect.com/science/article/B6WXH-4BT1SB3-1/2/d033257ac2462d714c4155e378292b73

Male and female of F344 rats were treated per os with nicardipine (Nic) and nifedipine (Nif), and changes in the levels of mRNA and protein of hepatic cytochrome P450 (P450) enzymes, CYP2B1, CYP2B2, CYP3A1, CYP3A2, CYP3A9, and CYP3A18 were examined. Furthermore, hepatic microsomal activities for pentoxyresorufin O-dealkylation (PROD) and nifedipine oxidation, which are mainly mediated by CYP2B and CYP3A subfamily enzymes, respectively, were measured. Analyses of RT-PCR and Western blotting revealed that Nic and Nif induced predominantly CYP3A and CYP2B enzymes, respectively. As for the gene activation of CYP2B enzymes, especially CYP2B1, Nif showed high capacity in both sexes of rats, whereas Nic did a definite capacity in the males but little in the females. Gene activations of CYP3A1, CYP3A2, and CYP3A18 by Nic occurred in both sexes of rats, although that of CYP3A9 did only in the male rats. Although gene activations of CYP3A1 and CYP3A2 by Nif were observed in both sexes of rats, a slight activation of the CYP3A9 gene occurred only in female rats, and the CYP3A18 gene activation, in neither male nor female rats. Thus, changes in levels of the mRNA or protein of CYP2B and CYP3A enzymes, especially CYP2B1 and CYP3A2, were closely correlated with those in hepatic PROD and nifedipine oxidation activities, respectively. The present findings demonstrate for the first time the sex difference in the Nic- and Nif-mediated induction of hepatic P450 enzymes in rats and further indicate that Nic and Nif show different specificities and sex dependencies in the induction of hepatic P450 enzymes.


http://www.sciencedirect.com/science/article/B6WXH-49NXGG3-4/2/57529a79531b012515320471dc3125c

We showed previously that lipopolysaccharide (LPS) induces noncholinergic airway hyperreactivity to capsaicin via an upregulation of tachykinin synthesis. This study was designed to test whether double-stranded preprotachykinin (ds PPT) RNA, RNA interference (RNAi), prevents the LPS-induced alterations. First, cultured primary nodose ganglial cells of newborn Brown-Norway rats were divided into four groups: control; LPS; LPS+RNAi; and LPS+RNAi+liposome. Second, young Brown-Norway rats for the in vivo study were divided into three groups (control; LPS; and LPS+RNAi), and ds PPT RNA was microinjected bilaterally into the nodose ganglia in the LPS+RNAi group. Then, ganglial cells were collected from the culture whereas the nodose ganglia and lungs were sampled from the animals, and PPT mRNA and substance P (SP) levels were analyzed. Also, airway reactivity to capsaicin was performed in vivo. LPS induced significant increases in PPT mRNA and SP levels in vitro and in vivo and an increase in airway reactivity to capsaicin in vivo. However, ds PPT RNA, but not scrambled RNA, prevented all LPS-induced alterations. The effect of ds PPT RNA was not enhanced by liposome in vitro. Therefore, we demonstrated that the local application of RNAi prevents effectively the activation of the noncholinergic system modulating the lungs/airways.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental toxin that induces hepatotoxicity and increases endotoxin-induced liver injury. The objective of this study was to evaluate whether TCDD could modulate apoptosis and cytokine-controlled apoptotic signaling pathways following lipopolysaccharide (LPS) exposure in female B6C3F1 mice. The effects of TCDD treatment were most dramatic late in the time course (10-14 days posttreatment). Serum enzyme activities were elevated at day 10 (100 [mu]g TCDD/40 [mu]g LPS treatment) and day 14 (100 [mu]g TCDD/saline treatment), indicating peak liver damage occurred at those times. Histological examination of perfused livers showed an increase in apoptotic cells at day 14 in animals treated with 10 [mu]g TCDD. Caspase-1 activity was suppressed at 14 days in mice treated with 100 [mu]g TCDD/40 [mu]g LPS and 100 [mu]g TCDD/4 [mu]g LPS compared to the respective corn oil (CO)/LPS-treated controls. Caspase-3 activity was suppressed at 14 days in 100 [mu]g TCDD/saline-100 [mu]g TCDD/40 [mu]g LPS- and 100 [mu]g TCDD/4 [mu]g LPS-treated mice compared to respective CO/saline- or CO/LPS-treated control mice. At 40 [mu]g LPS, caspase activity was stimulated in TCDD (100 [mu]g)-exposed mice at 3 and 7 days and then suppressed at 10 and 14 days. Western blot analysis, electrophoretic mobility shift assay, and ELISA did not show any effect by TCDD (100 [mu]g) on I[kappa]B-[beta] and I[kappa]B-[alpha] protein expression or on DNA binding activity of the nuclear NF[kappa]B protein. These data indicate that TCDD induces apoptosis 14 days posttreatment; however, we found no evidence of suppression of the antiapoptotic transcription factor NF[kappa]B.

Human paraoxonase (PON1) plays a role in detoxification of organophosphorus (OP) compounds by hydrolyzing the bioactive oxons, and in reducing oxidative low-density lipoproteins, which may protect against atherosclerosis. Some PON1 polymorphisms have been found to be responsible for variations in catalytic activity and expression and have been associated with susceptibility to OP poisoning and vascular diseases. Both situations are of public health relevance in Mexico. Therefore, the aim of this study was to evaluate PON1 phenotype and the frequencies of polymorphisms PON1 -162, -108, 55, and 192 in a Mexican population. The studied population consisted of unrelated individuals (n = 214) of either gender, 18-52 years old. Serum PON1 activity was assayed using phenylacetate and paraoxon as substrates. PON1 variants, -162, 55, and 192, were determined by real-time PCR using the TaqMan System, and PON1 -108 genotype by PCR-RFLP. We found a wide interindividual variability of PON1 activity with a unimodal distribution; the range of enzymatic activity toward phenylacetate was 84.72 to 422.0 U/mL, and 88.37 to 1645.6 U/L toward paraoxon. All four PON1 polymorphisms showed strong linkage disequilibrium (D% >90). PON1 polymorphisms -108, 55, and 192 were independently associated with arylesterase activity; whereas the activity toward paraoxon was related only with PON1 192 polymorphism, suggesting that this polymorphism is determinant to infer PON1 activity. A better understanding of the phenotype and genotypes of PON1 in Mexican populations will facilitate further epidemiological studies involving PON1 variability in OP poisoning and in the development of atherosclerosis.

CYP4B1 isoforms from rodents and other common laboratory animals are involved in the bioactivation of a range of protoxins, including 2-aminofluorene, 4-ipomeanol, and valproic acid. However, an earlier study provided evidence for a human allele encoding a nonfunctional CYP4B1 enzyme due to a Pro427Ser transversion in the meander region of the protein. In the present study, the CYP4B1 gene from several racial groups, Caucasians, African-Americans, and Hispanics, and from six nonhuman primate species was genotyped using a PCR-Hinf1 restriction enzyme fragment length polymorphism assay or by direct sequencing. All human populations examined were found to possess only the Ser allele at codon 427 (1279TCT) and all of the nonhuman primate species possessed only the Pro (CCT) allele. Therefore, an inactivating 1279C->T mutation in the human CYP4B1 gene likely arose following divergence of the Homo and Pan clades. Amino acid sequence alignments revealed further that this key Pro residue is located two amino acid residues N-terminal to the distal Arg of a Glu-Arg-triad thought to participate in heme binding and/or redox partner interactions. Mutation of the corresponding Arg424 residue in rabbit CYP4B1 to Leu, but not His, resulted in a loss of lauric acid hydroxylase activity and ability to generate a reduced-CO binding spectrum. These data provide additional evidence for the importance of this meander region Pro-X-Arg motif in CYP4B1 heme binding and catalytic function.

Toxicology in Vitro  (6)


Peroxisome proliferators comprise a group of structurally diverse chemicals which share as a common biologic effect the induction of peroxisomal fatty acid degrading enzymes. Concomitantly, the number and size of peroxisomes within hepatocytes increases. Following chronic administration some peroxisome proliferators act as non-genotoxic hepatocarcinogens in susceptible species such as rodents. To establish an in vitro model for the toxicological investigation of peroxisome proliferation, primary hepatocytes of rats, dogs and humans were cultivated in an organotypic cell culture model (sandwich model). By employing a panel of diverse compounds in this model a graded response was observed in the induction of carnitine acetyl transferase (CAT), the activity of which was determined as an endpoint. The following results were obtained in the order of decreasing inducing potential for rat hepatocytes: FOE 3798>nafenopin>fenofibrate (ciprofibrate>bezafibrate >> DEHP[ap]ETYA>DEHA. Induction of CAT activity was generally higher than reported in earlier cell culture systems, probably reflecting the effect of the extracellular matrix provided by the collagen gel sandwich. In parallel, transcription of the rat CYP4A1 gene was induced by a similar order of magnitude as measured by TaqMan RT-PCR. In accordance with literature data, human and dog hepatocytes did not display such a strong and graded response but rather were not susceptible to this effect. In addition, 3H-thymidine incorporation data demonstrated that nafenopin was able to induce DNA synthesis in rat hepatocytes whereas it did not in human hepatocytes.

In order to study the influence of temperature on vitellogenin gene and estrogen receptor gene expression in primary hepatocytes from rainbow trout (Oncorhynchus mykiss), cells were exposed to 17[beta]-estradiol, bisphenol-A and nonylphenol for 48 and 96 hr. Induction of vitellogenin-mRNA expression was detected in a non-radioactive dot blot/RNAse protection assay and by RT-PCR. In the dot blot/RNAse protection assay, the estrogenic potentials of bisphenol-A and nonylphenol were about 104- to 105-fold and 105-fold lower than that of 17[beta]-estradiol, respectively. The relative estrogenic potential did not show any difference between 14 and 18[deg]C. In contrast, at 18[deg]C, RT-PCR analysis revealed increased amounts of vitellogenin- and estrogen receptor-mRNA after 12 and 24 hr of exposure to 17[beta]-estradiol, if compared to 14[deg]C. Owing to increased vitellogenin gene expression at 18[deg]C, the sensitivity of primary hepatocytes to 17[beta]-estradiol and bisphenol-A could be increased.


It has been reported previously that in vitro treatment of human blood derived dendritic cells (DC) with contact allergens provokes the elevated expression of mRNA for interleukin (IL) 1[beta], under conditions where similar treatment of cells with the non-sensitizing skin irritant sodium lauryl sulfate (SLS) did not alter IL-1[beta] mRNA levels (Reutter et al., 1997). The purpose of the present investigation was to evaluate further this phenomenon and to explore the potential utility of this approach for the purpose of skin sensitization testing. Human peripheral blood progenitor cells prepared from healthy adult volunteers were cultured in the presence of IL-4 and granulocyte/macrophage colony stimulating factor. After 5 days of culture, the majority of cells had a Langerhans cell-like phenotype, with characteristic dendritic morphology and cell surface expression of CD83, major histocompatibility complex class II and CD1a determinants. These blood-derived DC were cultured in the presence of the contact allergen 2,4-dinitrofluorobenzene (DNFB), SLS or vehicle alone and mRNA expression for IL-1[beta], IL-6 and IL-18 was analysed by semiquantitative reverse transcriptase polymerase chain reaction. Constitutive expression of all three cytokines was observed for DC isolated from all donors examined. Exposure to DNFB resulted in upregulation of IL-1[beta] mRNA (two- to threefold) in cells derived from three out of eight donors whereas IL-6 and IL-18 were largely unaffected by allergen exposure. In contrast, SLS treatment did not induce IL-1[beta] mRNA expression in any of the donors investigated. Analysis of cytokine mRNA expression using the protocol described by Reutter et al. (1997), did not increase the sensitivity of measurement of induced cytokine expression. Although selected upregulation of IL-1[beta] by blood derived DC has been confirmed, a wider range of contact allergens and irritants need to be assessed before this approach could be considered for hazard identification.

Epidermal mRNA for interleukin 1[beta] (IL-1[beta]) has been shown to be increased following exposure of mouse skin to sensitizing compounds. In addition, this early upregulation of IL-1[beta] was specific for contact sensitizers, while expression of IL-1[beta] was unaffected by irritants. Langerhans cells are the major source of IL-1[beta] within the epidermis in the induction phase of skin sensitization. Since the isolation of Langerhans cells from skin biopsies results only in low frequencies, we decided to use dendritic cells (DCs) generated from peripheral blood as Langerhans cell equivalents to investigate the ability of five contact sensitizers and one irritant to induce IL-1[beta] gene expression in vitro. For our studies we cultivated DCs in serum-free medium supplemented with granulocyte/macrophage-colony stimulation factor (GM-CSF) and interleukin 4 (IL-4). The DCs showed a typical dendritic morphology, a characteristic expression of surface markers and high stimulatory capacity for autologous T cells. 5-day-old DCs were incubated with subtoxic concentrations of the contact sensitizers pentadecyl-catechol, 2,4,6-trinitrobenzene sulfonic acid, 2,4-dinitrofluorobenzene, NiSO4, K2Cr2O7 and the irritant sodium dodecyl sulfate. IL-1[beta] mRNA expression was detected by using the reverse transcriptase-polymerase chain reaction (RT-PCR) technique and non-radioactive hybridization procedures. For all contact sensitizers, expression of IL-1[beta] mRNA increased, whereas treatment with the irritant SDS had no significant effect on IL-1[beta] expression. Thus we developed an in vitro system, which may be useful to evaluate allergic potentials of chemicals and products.


Bisphenol A, an endocrine-disrupting chemical, is widely used in many consumer products. We previously showed the sulfoconjugation of bisphenol A catalyzed by a human thermostable phenol sulfotransferase, ST1A3. The estrogenic potency of bisphenol A sulfate was compared with that of bisphenol A by an E-screen assay using human breast cancer MCF-7 cells. An increase in the expression level of an estrogen-responsive pS2 gene was also examined using MCF-7 cells after exposure to bisphenol A and its sulfate for their estrogenicity. Bisphenol A sulfate did not exhibit estrogenic effects at 0.1 [μ] (E-screen assay) and 1 m (pS2 gene expression) compared with bisphenol A, which exhibited the effects at 3 n (E-screen assay) and 1 [μ] (pS2 gene expression), respectively. We have therefore evaluated major roles of cytosolic phenol sulfotransferase in the human liver. Bisphenol A sulfation in human liver cytosols was inhibited by more than 90% by p-nitrophenol and quercetin, a typical substrate and specific inhibitor of phenol sulfotransferase, respectively. These results indicated that the estrogenicity of bisphenol A was abolished through its sulfation catalyzed by a human hepatic thermostable phenol sulfotransferase.

capacity to differentiate in vitro into a wide variety of cell types. Based on this potential the embryonic stem cell test (EST) has been developed, which represents an assay system for the classification of compounds for their teratogenic potential, based on the morphological evaluation of contracting myocard cells compared to the cytotoxic effects on undifferentiated stem cells and adult 3T3 fibroblasts. To expand the EST, the quantitative expression of the [alpha]- and [beta]-myosin heavy chain (MHC) genes under the influence of test compounds was studied employing real-time TaqMan PCR analysis. The molecular evaluation of the MHC genes allows a higher sensitivity for the classification of substances and the transfer of the EST to the molecular level allows to start experimental procedures at day 9 of culture. Thus, the modulated EST holds promise as a new easily quantifiable in vitro screening assay in teratology.

Toxicology Letters (8)


http://www.sciencedirect.com/science/article/B6TCR-4C47J23-6/2/d4fefa5499232039717baa44b805331c

Occupational exposures have long been suspected to play a role in the incidence of renal cell carcinoma (RCC). Especially, the carcinogenicity of the industrial solvent trichloroethylene (TCE) has been controversially debated, both with respect to the epidemiological and the molecular studies. In order to further elucidate this issue, it appeared important to compare suitable RCC patient groups, i.e., TCE-exposed versus non-TCE-exposed patients. We evaluated RCC from a previous German study that had described differences in RCC risks between TCE-exposed (n=17) and non-exposed patients (n=21). We compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the kidney cancer causing VHL tumor suppressor gene. RCC did not differ with respect to histopathological characteristics in both patient groups. We noticed a younger age at diagnosis in TCE-exposed patients compared to non-exposed patients (P=0.01). Moreover, the non-TCE-exposed patients did not share the somatic VHL mutation characteristics of TCE-exposed patients such as the previously identified hot spot mutation 454 C > T P81S or multiple mutations. These data support the notion of a putative genotoxic effect of TCE leading to VHL gene damage and subsequent occurrence of RCC in highly exposed subjects.


http://www.sciencedirect.com/science/article/B6TCR-44JHY8R-7/2/fd4cbecc082de4a2bde6ac0d1d7f0139

Cadmium (Cd+2) has been shown to transiently increase the expression of mRNA for the third isoform of the metallothionein (MT-3) gene family in cultured human proximal tubule (HPT) cells. The goal of the present study was to further define the expression of MT-3 in mortal (HPT) and immortal (HK-2) cultures of HPT cells when exposed to lethal and sub-lethal concentrations of Cd+2 under both acute and chronic time periods of exposure. Expression of MT-3 mRNA and
protein was determined in cultured HPT cells and HK-2 cells using reverse-transcription-polymerase chain reaction (RT-PCR) and immuno-blotting, and expression of c-fos, c-jun and c-myc mRNA by RT-PCR. The results confirmed that exposure of the HPT cells to Cd+2 induced a transient increase in MT-3 mRNA and extended the induction to include a subsequent transient increase in the level of the MT-3 protein. The induction of MT-3 was rapid and returned to control values within 48 h of exposure despite the continued presence of lethal and sublethal concentrations of Cd+2. It was also demonstrated that the pattern of expression of MT-3 mRNA was similar to that of the early response genes, c-fos, c-jun and c-myc. It was shown that the HK-2 cells did not express MT-3 when exposed to Cd+2, but had similar expression of the c-fos, c-jun and c-myc genes. The results demonstrate that MT-3 expression is metal responsive in HPT cells.


Changes in the gene expressions of hepatic enzymes responsible for cholesterol homeostasis were examined during the process of lead nitrate (LN)-induced development of hypercholesterolemia in male rats. Total cholesterol levels in the liver and serum were significantly increased at 3-72 h and 12-72 h, respectively, after LN-treatment (100 [mu]mol/kg, i.v.). Despite the development of hypercholesterolemia, the genes for hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and other enzymes (FPPS, farnesyl diphosphate synthase; SQS, squalene synthase; CYP51, lanosterol 14[alpha]-demethylase) responsible for cholesterol biosynthesis were activated at 3-24 h and 12-18 h, respectively. On the other hand, the gene expression of cholesterol 7[alpha]-hydroxylase (CYP7A1), a catabolic enzyme of cholesterol, was remarkably suppressed at 3-72 h. The gene expression levels of cytokines interleukin-1[beta] (IL-1[beta]) and TNF-[alpha], which activate the HMGR gene and suppress the CYP7A1 gene, were significantly increased at 1-3 h and 3-24 h, respectively. Furthermore, gene activation of SREBP-2, a gene activator of several cholesterogenic enzymes, occurred before the gene activations of FPPS, SQS and CYP51. This is the first report demonstrating sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis in LN-treated male rats. The mechanisms for the altered-gene expressions of hepatic enzymes in LN-treated rats are discussed.

especially Pi*Z, could help to predict asbestosis risk and confirm the high prevalence of the Pi*S allele in Spain.


http://www.sciencedirect.com/science/article/B6TCR-3VXBPDC-V/2/c3c8477efeb17707e3b0f904ac98b5ef5

Our previous observations on the toxic effects of hydroxyurea (HU) in adrenalectomized (ADX) rats prompted us to suggest that these effects might be mediated by an increased synthesis of proinflammatory cytokines. This study was conducted to determine whether HU stimulates cytokine gene expression in vivo. The polymerase chain reaction (PCR) technique was used to assess levels of mRNA for interleukin-1[alpha] (IL-1[alpha]), tumor necrosis factor (TNF) and interleukin-4 (IL-4) in spleen cells from intact and ADX rats treated with HU or vehicle. In ADX rats, expression of proinflammatory-cytokine mRNA was markedly increased by HU, but no expression of these genes was seen in intact animals after treatment. In the latter rats, cytokine-gene expression seemed to be down-regulated by HU-induced elevations in plasma corticosterone levels, since IL-1[alpha] and TNF transcripts could be detected only after corticosterone levels had returned to normal (24 h after treatment). Interestingly, IL-4 mRNA could not be detected in either treated or untreated ADX rats, indicating that expression of this gene is closely related to circulating levels of corticosterone. These findings strongly suggest that the increased toxicity displayed by HU in ADX animals is mediated by stimulation of cytokine synthesis in vivo.


http://www.sciencedirect.com/science/article/B6TCR-408BJ93-5/2/df3d60f0a9885c36f451c52a8

The mitochondrial toxin 3-nitropropionic acid (3-NPA) causes neurodegeneration in the basal ganglia and neurological symptoms resembling Huntington's disease (HD) when applied to primates or rodents, and therefore might be used as an animal model for this disorder. For that reason, the molecular mechanisms involved in 3-NPA-induced neurodegeneration are of considerable interest. In our model, murine neuroblastoma cells (Neuro-2a) were treated with different doses of 3-NPA, and changes in gene expression were analyzed by means of mRNA differential display (DDRT-PCR). Using 18 primer combinations, we have identified a set of 33 candidate cDNAs deriving from 29 excised DDRT bands whose expression appeared to be changed in response to the 3-NPA insult (mostly elevated). DNA sequencing revealed that novel, as well as previously described genes, are included in this panel. Amongst the known cDNAs, the differential mRNA expression of the ribosomal proteins S6 and L40, of the protein kinase A (PKA) catalytic beta subunit and of the intercellular adhesion molecule ICAM-1 could be verified using Northern hybridization and RT-PCR, respectively. Furthermore, ICAM-1 expression could also be shown to increase at the protein level, which points to a possible function for this molecule in neuronal cells in the course of neurodegeneration. The results may prove useful in elucidating the multiple processes causing neurodegeneration subsequent to lesions by mitochondrial toxins and excitotoxins as well.

http://www.sciencedirect.com/science/article/B6TCR-460DFXH-1/2/f2249d82b2c2600a64d52706a2d75616

The expression of heat shock protein (hsp) 90[alpha] and [beta] mRNA and protein were determined in the human kidney and in human proximal tubule (HPT) cells exposed to lethal and sub-lethal concentrations of Cd+2 under both acute and extended conditions of exposure. Using immunohistochemical analysis, it was demonstrated that hsp 90 was widely distributed in the human adult and fetal kidney. Moderate to strong staining was observed in the straight portions of the distal and proximal tubules, the distal convoluted tubule, the collecting ducts and the parietal epithelium of Bowmans capsule in the glomerulus. Moderate staining was observed in the proximal convoluted tubule of the cortex and the thick loops of Henle within the medulla. In addition, the fetal kidney demonstrated strong staining of the blastema, the 'S-shaped' bodies, and the developing glomeruli. Analysis of hsp 90[alpha] and [beta] mRNA expression in total RNA isolated from in situ microdissected proximal tubules or HPT cells demonstrated similar expression levels of both the [alpha] and [beta] isoforms in this tubule segment. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (NaAsO2). Heat stress, elevated temperature at 42.5 [deg]C for 1 h, caused a modest increase in both hsp 90[alpha] and [beta] mRNA and protein. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M NaAsO2 for 4 h. In contrast, acute exposure of HPT cells to 53.4 [mu]M CdCl2 for 4 h resulted in no consistent increase in hsp 90[alpha] and [beta] mRNA or protein. Chronic exposure to Cd+2 likewise failed to increase either hsp 90 mRNA or protein expression, even at concentrations of Cd+2 that were lethal to the cells during the time course. This study shows that the HPT has a high basal expression of hsp 90, which is not induced by Cd+2 exposure.


http://www.sciencedirect.com/science/article/B6TCR-408BJC1-5/2/e344ad1d859faea13b8624a03df873dd

The expression of hsp 60 mRNA and protein were determined in human proximal tubule cells (HPT) exposed to lethal and sub-lethal concentrations of Cd2+ under both acute and extended conditions of exposure. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (sodium arsenite). Heat stress, elevated temperature at 42.5[deg]C for 1 h, caused an increase in both hsp 60 mRNA and protein following removal of the stress. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M sodium arsenite for 4 h. Acute exposure of HPT cells to 53.4 [mu]M CdCl2 for 4 h also resulted in an increase in hsp 60 mRNA and protein following removal of the metal. An extended exposure to Cd2+ was modeled by treating the cells continuously with Cd2+ at both lethal and sub-lethal levels over a 16-day time course. It was demonstrated that chronic exposure to Cd2+ failed to increase either hsp 60 mRNA or protein expression in HPT cells, even at concentrations of Cd2+ that were lethal to the cells during the time course. In fact, hsp 60 protein levels were decreased compared to controls at lethal levels of Cd2+ exposure. These findings suggest that hsp 60 expression may have two distinct roles when the human proximal tubule cell is exposed to Cd2+. A protective role through hsp 60 induction when the proximal tubule cell is acutely exposed to Cd2+ and a deleterious role when hsp 60 protein is down-regulated during extended exposure to Cd2+. 

http://www.sciencedirect.com/science/article/B6TCS-43K2G13-G/2/04a86f0b5a781c7f1a11bb747bddd3512

The release of pro-inflammatory cytokines (IL-1[beta], IL-6 and TNF-[alpha]) from murine peritoneal adherent cells (MPAC) was studied after exposure to jararhagin, a metalloproteinase/disintegrin isolated from Bothrops jararaca venom. MPACs were treated with LPS (lipopolysaccharide), jararhagin, or EDTA-inactivated jararhagin for up to 24 h. Following incubation, the culture supernatant was assayed by ELISA for the presence of cytokines, while the cells were analysed for viability and cytokine mRNA expression. The cells exposed to native jararhagin released TNF-[alpha] and IL-1[beta] after 4 and 24 h respectively. When MPACs were exposed to Jararhagin treated with EDTA, TNF-[alpha] and IL-1[beta] production was sustained throughout the culture period and IL-6 production was observed. TNF-[alpha], IL-6 and IL-1[beta] mRNA were detected 4 h after stimulation with either native or EDTA-treated jararhagin. Addition of jararhagin to LPS stimulated cells resulted in a dramatic decrease in the release of IL-6 and TNF-[alpha]. RT-PCR showed that this inhibition does not occur at the transcriptional level and further experiments showed that jararhagin degraded soluble cytokines by proteolytic activity. This study suggests that jararhagin induces TNF-[alpha], IL-1[beta] and IL-6 expression, which may be rapidly degraded by its proteolytic activity.


http://www.sciencedirect.com/science/article/B6TCS-48KF64N-1/2/4d3374021a5e286c7c37ba59eb8ef6e1

Aerosol exposure to ricin causes irreversible pathological changes of the respiratory tract resulting in epithelial necrosis, pulmonary edema and ultimately death. The pulmonary genomic profile of BALB/c mice inhalationally exposed to a lethal dose of ricin was examined using cDNA arrays. The expression profile of 1178 mRNA species was determined for ricin-exposed lung tissue, in which 34 genes had statistically significant changes in gene expression. Transcripts identified by the assay included those that facilitate tissue healing (early growth response gene (egr)-1), regulate inflammation (interleukin (IL)-6, tristetraproline (ttp)), cell growth (c-myc, cytokine-inducible SH2-containing protein (cish)- 3), apoptosis (T-cell death associated protein (tdag)51, pim-1) and DNA repair (ephrin type A receptor 2 (ephA2)). Manipulation of these gene products may provide a means of limiting the severe lung damage occurring at the cellular level. Transcriptional activation of egr-1, cish-3, c-myc and thrombospondin (tsp)-1 was already apparent when pathological and physiological changes were observed in the lungs at 12 h postexposure. These genes may well serve as markers for ricin-induced pulmonary toxicity. Ongoing studies are evaluating this aspect of the array data and the potential of several genes for clinical intervention.

http://www.sciencedirect.com/science/article/B6TCS-44HTMX5-1/2/07784f5c6b087787f50076c99156cd40

The neurotoxic activity of ammodytoxin A (AtxA), a phospholipase A2 from Vipera ammodytes ammodytes venom, has been investigated by protein engineering. With the aim of obtaining AtxA as a non-fused protein in the bacterial cytoplasm and avoiding problems with incomplete cleavage in vivo of the initial Met preceding the first residue (Ser1), a double mutant (S1A/E4Q) was prepared and expressed in Escherichia coli. Immunoblotting of the bacterial lysate showed that the mutant was synthesized at a low level not exceeding 0.5% of total cell protein. Analysis of the potential secondary structure of the mutant mRNA in the translation initiation region suggested that the Ala1 (GCC) and Leu2 (CUG) codons used are likely to be involved in a hairpin structure with the Thr13 (ACG) and Gly14 (GGG) codons, hindering effective translation at the ribosome. To weaken this structure (by [Delta]G of about 20 kJ/mol) the same double mutant was prepared using another mutagenic oligonucleotide with silent mutations in the Ala1 (GCU) and Leu2 (UUG) codons. The mutant was successfully produced at a level of approximately 15% of total protein, with the initial Met completely removed in the bacterial cell. Such an approach could be important in solving similar problems in bacterial production of other toxic proteins.


http://www.sciencedirect.com/science/article/B6TCS-49HMS1P-1/2/1204fc67d1441720fc61488796a69425

The Thalassophryne nattereri fish venom induces a severe burning pain, oedema, and necrosis observed both clinically and experimentally. The present study was carried out in order to describe the pattern of local acute inflammatory response after T. nattereri venom injection. Our findings show that the edematogenic response induced by T. nattereri venom in footpad of mice was dose- and time dependent, and remained significantly elevated over 48 h after injection. Analysis of footpad homogenates were tested for the presence of TNF-[alpha], IL-1[beta] and IL-6, and demonstrated augmented levels of these cytokines. Our results showed that the injection of venom developed an inadequate cellular inflammatory response evidenced by poor infiltration of mononuclear cells, preceded by decreased number of these cells in peripheral blood. In contrast, we observed an early intense recruitment of neutrophil to peritoneal cavity, accompanied by a significant decrease in the number of mononuclear cells. A drastic increase in the total amount of cells, mainly in neutrophils, followed by mononuclear cell recruitment was observed 24 h. In addition, we also demonstrated that T. nattereri venom affects the viability of mononuclear cells (J774A1) in culture. We conclude that the scarcity of inflammatory cellular influx into local lesions (intraplantar) induced by T. nattereri venom could be a consequence of an impaired blood flow in venules at injured tissue and cytotoxic effect of the venom on inflammatory cells can contribute to this impairment.

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http://www.sciencedirect.com/science/article/B75GP-4C0DW4J-26P/2/adab96c3a385f65ecfb3488c9d224ecb

Mixed malaria infections (Plasmodium falciparum and P. vivax) are suspected to occur at a greater frequency than is detected by conventional light microscopy. To determine this frequency we carried out a prospective 'blinded' comparison of diagnosis by conventional light microscopy and enzymatic amplification of the circumsporozoite gene extracted from dried spotted blood samples. Patients were previously healthy, active duty Thai soldiers assigned to a malaria risk area presenting with malaria. Microscopy (oil immersion objective at 1000 x magnification) involved examination of Giemsa-stained thick and thin blood films by an experienced microscopist. Whole blood samples (25 [mu]l) dried on filter paper were used for species-specific parasite deoxyribonucleic acid (DNA) amplification by the polymerase chain reaction (PCR) and hybridization with radiolabelled P. falciparum and P. vivax probes. Of 137 Consecutive cases of malaria studied, 9% (3/32) of microscopically diagnosed P. falciparum infections and 5% (5/104) of microscopically diagnosed P. vivax infections were found to be mixed by the PCR/DNA probe systems, While 1 Case was diagnosed as mixed by both microscopy and PCR. The possibility that malaria patients may have undetected mixed infections should be kept in mind because of the specific therapy required both for P. falciparum and for radical cure of P. vivax.


http://www.sciencedirect.com/science/article/B75GP-4DKTH96-GK/2/0600cc8c157753876fcd336255bf667b

In Liberia, little information is available on the efficacy of antimalarials against Plasmodium falciparum malaria. We measured parasitological resistance to chloroquine and sulfadoxine-pyrimethamine (SP) in Harper, south-west Liberia in a 28-d study in vivo. A total of 50 patients completed follow-up in the chloroquine group, and 66 in the SP group. The chloroquine failure rate was 74.0% (95% confidence interval [95% CI] 59.7-85.4%) after 14 d of follow-up and 84.0% (95% CI 70.9-92.8%) after 28 d (no polymerase chain reaction [PCR] analysis was performed to detect reinfections in this group). In the SP group, the failure rate was 48.5% (95% CI 36.2-61.0%) after 14 d and 69.7% (95% CI 57.1-80.4%) after 28 d, readjusted to 51.5% (95% CI 38.9-64.0%) after taking into account reinfections detected by PCR. Genomic analysis of parasite isolates was also performed to look for point mutations associated with resistance. Genotyping of parasite isolates revealed that all carried chloroquine-resistant K-76T mutations at gene pfcr, whereas the triple mutation (S108N, N511, C59R) at dhfr and the A437G mutation at dhps, both associated with resistance to SP, were present in 84% and 79% of pretreatment isolates respectively. These results seriously question the continued use of chloroquine and SP in Harper and highlight the urgency of making alternative antimalarial therapies available. Our study confirms that resistance to chloroquine may be high in Liberia and yields hitherto missing information on SP.

The 2 known host trees of Cryptococcus neoformans var. gattii, Eucalyptus camaldulensis and E. tereticornis, do not occur naturally in the 'Top End' of the Northern Territory (NT) of Australia. Nine clinical isolates of C. neoformans var. gattii from the NT were analysed by random amplification of polymorphic deoxyribonucleic acid and polymerase chain reaction 'fingerprinting'. Two isolates were assigned to profile VGI, previously established as the common RAPD profile. The remaining 7 were assigned to profile VGII; 6 of these isolates were recovered from individuals living in the 'Top End'. The results strongly support the existence of an alternative environmental niche for C. neoformans var. gattii, as all isolates from Eucalyptus spp. in Australia to date have been of profile VGI.


Polymerase chain reaction (PCR) is now widely used in malaria research for analysis of field samples. However, little has been reported regarding loss of sensitivity due to field methodology. Therefore, studies were carried out in relation to blood sampling (anticoagulants, culture medium, filter paper), storage (temperature, time and immediate lysis) and handling (repeated thawing and freezing). The PCR was unaffected by citrate and EDTA but partly inhibited by heparin (inhibition was reversed by heparinase at optimal concentrations). Samples collected on filter paper showed a significant 100-fold lower sensitivity (compared to control samples frozen immediately after collection) when stored at 30 [deg]C and 60% humidity; and the paper quality appeared to be critical. Storage of unprocessed whole blood at 4 [deg]C, 20 [deg]C or 30 [deg]C rarely resulted in any loss of sensitivity. Repeated thawing generally resulted in 10-fold loss of sensitivity compared to blood kept frozen until DNA extraction. The presence of antimalarial drug did not apparently affect sensitivity. We conclude that the mode of collection and storage of blood samples may influence the sensitivity of detection of malaria parasites by PCR. This may be critical in studies including individuals with low parasitaemia, mixed infections and comparison of data from different settings.


To assess the utility of single-stranded conformational polymorphism (SSCP) analysis for the differentiation of schistosomes, using methods adapted for a Perkin Elmer ABI Prism 377(TM) automated sequencer, 3 isolates of Schistosoma haematobium, 2 of S. intercalatum and single isolates of S. curassoni and S. bovis were selected for study. Two fluorescently labelled, double-stranded polymerase chain reaction products, amplified from the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and the nuclear ribosomal second internal transcribed spacer (ITS2), were generated from single male and female worms. Changes in electrophoretic mobility of fragments within an SSCP profile revealed variation at individual, isolate and species levels. The mutational basis between representative SSCP profiles was confirmed by direct sequencing, demonstrating that single point substitutions were detectable. SSCP analysis has considerable potential as an alternative molecular method of identification and characterization of schistosomes. More broadly, fluorescence-based SSCP analysis is applicable to almost any gene target from any species of parasite and is a powerful molecular tool for genetic profilling.


http://www.sciencedirect.com/science/article/B75GP-4BY30RJ-11/2/00103d4b3d390b88be4c69468529104c73

Before beginning treatment for cutaneous leishmaniasis, parasitological confirmation of the disease is required. The most commonly used diagnostic procedures are microscopy and culture of samples taken from the active edge of the lesion. In this study, we compared the sensitivity of previous diagnostic procedures with the polymerase chain reaction (PCR), using smears taken from the edge of the lesion and its centre. The sensitivity was greater with smears taken from the centre of the lesion, both for microscopical examination (85%) and for PCR (81%), compared to those obtained from the edge of the lesion (69% and 58% respectively). When PCR was carried out on biopsy material from the edge of the lesion the sensitivity was 63%.


http://www.sciencedirect.com/science/article/B75GP-4D33HTG-50/2/c09c32004f9d8437647d735daa482b76

Single-strand conformation polymorphism (SSCP) analysis was employed for the direct visual display of genetic variability in mitochondrial DNA (mtDNA) fragments within and among populations of Echinococcus granulosus from the People’s Republic of China and from Argentina. Fragments of the NADH dehydrogenase I gene (NDI) and the cytochrome c oxidase subunit I (COI) were individually amplified from parasite DNA by polymerase chain reaction, denatured and subjected to SSCP analysis. Using NDI and COI fragments, samples representing different genotypes could be readily identified based on characteristic SSCP profiles. The results demonstrate the utility of SSCP for the direct visual display of nucleotide variation in mtDNA of E. granulosus prior to DNA sequence analysis. The approach compares favourably with existing genotyping procedures and provides a reliable and technically reproducible method for the routine laboratory identification of Echinococcus isolates.

http://www.sciencedirect.com/science/article/B6W7X-43K2PMM-F/2/493f7b1827cca548500415d64502cec8


http://www.sciencedirect.com/science/article/B6TCT-3WK3RR8-3/2/2bf9a4fe0dfe4bed917a5aac1e99cee2

Leukocyte depleted blood components are frequently used to reduce alloimmunization and the risk of transfusion transmitted infection. Counting residual white blood cells in filtered blood products requires sensitive and reliable techniques. After separation of white blood cells from 500 [mu]l of 20 non-filtered and 54 filtered blood products we used polymerase chain reaction (PCR) and fluorimetric detection for the quantification of genomic DNA. The results were compared with results from Nageotte chamber counting. The accurate limit of detection of PCR was determined at 1 WBC/[mu]l (intra-assay coefficient of variation: 16.3%). PCR correlated well with Nageotte chamber counts (r=0.77, p n=74). Concordant results were obtained in 51 filtered and 20 non-filtered blood products. Discrepant results were obtained in 3 filtered whole blood units: In these blood products >12 WBC/[mu]l were counted in Nageotte chamber and PCR gave a negative result. After component preparation fresh-frozen plasma and red cell concentrates of these units contained <1 WBC/[mu]l using both methods. In conclusion we describe a quantitative PCR method which had about the same sensitivity and specificity as Nageotte chamber testing. However, PCR is more laborious than the standard method. As well, as reliable PCR testing requires expensive instruments and staff experienced in molecular biology, the standard method is more cost effective.


http://www.sciencedirect.com/science/article/B6W6V-436FSD6-
Interleukin-12 (IL-12) is a heterodimeric cytokine implicated in the early differentiation of naive T-lymphocytes into the Th1 subset. IL-12 is important for induction of the cellular immune response against viruses, intracellular parasites and neoplasms. Its role in alloresponsiveness has not been fully elucidated. Preliminary data in the literature point toward the prevalence of Th1 lymphocytes in processes of allograft rejection. In attempt to further investigate the expression of this cytokine during episodes of cellular rejection of renal allografts, we searched for IL-12 message in human kidney allograft biopsies using the reverse transcriptase-polymerase chain reaction technique.

Twenty-three allograft core biopsies from 19 patients were obtained precutaneously for clinical indications in 18 cases, and as part of an investigational protocol in five cases. A portion of the tissue was used for RNA extraction using the guanidium-thiocyanate phenol-chloroform method. Histology was performed on the remaining core material. Ten mg of total RNA were used for reversed transcription. PCR of the c-DNAs was done for 40 cycles using primers for the p40 subunit of IL-12 and GAPDH which was used as a control. PCR products were photographed after electrophoresis, transferred to a nylon membrane and hybridized with a radiolabelled cloned human IL-12 p40 1 kb c-DNA fragment. Autoradiographies were developed after 20-min exposure. All samples were run in triplicate. IL-12 p40 m-RNA was expressed in all 17 biopsies showing acute cellular rejection as well as in all three biopsies showing focal interstitial fibrosis. No message was found in the presence of normal allograft histology. This is the first in vivo report of IL-12 p40 subunit m-RNA expression during renal allograft rejection in humans. The role of this Th1 cytokine in the alloresponse deserves further investigation.


http://www.sciencedirect.com/science/article/B6W6V-436FRRN-1B/2/30f98d3a8b21b5eab25ea9475255db2f

Porcine-to-human xenotransplantation offers a potential solution to the critical shortage of human organs. The major immunological barrier to xenotransplantation between these species is a rapid rejection process mediated by preformed natural antibodies and complement. Xenogeneic organ grafts are especially susceptible to complement mediated injury because complement regulatory proteins, which ordinarily protect cells from inadvertent injury during the activation of complement, function poorly in regulating activation of heterologous complement. Removal of xenoreactive antibodies or systemic inhibition of complement activity has been shown to prolong graft survival. As an alternative to the systemic inhibition of complement activity, we have established a model system using transgenic animals to test whether the expression of human membrane bound complement regulatory proteins on mouse endothelial cells can inhibit the activation of human complement. CD59, which acts at the terminal stage of complement activation by inhibiting the formation of the membrane attack complex, was used as a paradigm for this model. A CD59 construct containing the putative CD59 gene promoter linked to the CD59 coding region was used to demonstrate expression of the human CD59 protein in various tissues of transgenic mice, including endothelial cells in the heart. In addition, we show that the transgenic CD59 protein is biologically active as determined by the ability to inhibit the formation of membrane attack complex in transgenic mouse hearts perfused ex vivo with human plasma. These results demonstrate that expression of membrane bound complement regulatory proteins can achieve complement inhibition in a xenogeneic organ and suggest that this approach may be useful for successful xenotransplantation between discordant species.

Background: In a previous study it was shown that pre-transplant blood transfusion was associated with a better clinical outcome after heart transplantation (HTx). In this study the effect of heart transplantation (HTx) on the T cell receptor V beta chain (TCR[V[beta]]) repertoire was investigated. Therefore, we analyzed the TCR[V[beta]] repertoire of patients after HTx to see whether a correlation with clinical outcome could be observed. Methods: Patients were analyzed at four different time points: pre-HTx, less than 1 month post-HTx, between 1 month and 2.5 months post-HTx and more than 2.5 months post-HTx. CD4+ and CD8+ T cells were purified from patient peripheral blood mononuclear cells (PBMC). TCR beta chain usage was analyzed semiquantitatively by Southern blot analysis. Results: HTx affected the TCR[V[beta]] repertoire in both the CD4+ and CD8+ T cell compartments in all patients. Changes in the TCR[V[beta]] repertoire were most pronounced within the CD8+ T cell subset. Interestingly, one patient showed modulation in TCR[V[beta]] chain usage predominantly in the CD4+ T cell compartment. Conclusions: Modulation of TCR[V[beta]] chain usage was detected in all patients analyzed. No clear-cut relation was observed between TCR[V[beta]] modulation after transplantation and clinical outcome. In some cases modulations appeared to concur with observed immunological events (clinically and/or in-vitro).


Acute cellular allograft rejection is characterized by leukocyte invasion and tissue destruction, associated with qualitative and quantitative alterations in the extracellular matrix (ECM) compartment. Metabolism of ECM proteins is mainly regulated by matrix metalloproteinases (MMP), that are zinc depended endoproteinases. MMP, especially basement membrane degrading MMP-2 and MMP-9, also facilitate tissue invasion of leukocytes. In addition, MMP-2 exerts a direct pro-inflammatory effect upon glomerular mesangial cells. Therefore, the investigation of the role of MMP in transplant rejection may lead to novel approaches in the therapy of rejection processes. To our knowledge, this is the first study of acute allograft rejection, formally addressing expression and activity of MMP, including the effect of a MMP inhibiting agent. For our studies, we used the orthotopic kidney allograft model in the stringent Dark Agouti-to-Lewis rat strain combination. Animals were divided into four groups: group A, healthy untreated Lewis rats (n=3); group B, sham operated Lewis rats (n=3); group C, transplanted Lewis rats treated with vehicle solution only (n=12); group D, transplanted Lewis rats treated with MMP inhibitor BB-94 (n=12). Respective animals were treated once daily intraperitonealy with BB-94 (30 mg/kg) or vehicle solution only. Treatment lasted from the third preoperative day until the end of the experiment, the time of severe rejection at day +7. Acute kidney allograft rejection led to alterations in the expression and activity of MMP. Overall MMP activity slightly increased despite severe destruction of kidney histology. The MMP inhibitor BB-94 successfully inhibited MMP activity to a high extent. MMP expression did not show uniform findings, since acute rejection led to differential expression of MMP-2 and MMP-9. During the rejection process, MMP-9 showed a small but significant increase, whereas MMP-2 production decreased substantially. Interestingly, BB-94 was able to keep proteinuria at a low level in transplanted animals. In conclusion, MMP--especially MMP-9--appear to represent new mediators involved in acute kidney transplant rejection.

http://www.sciencedirect.com/science/article/B6W6V-4DBJ85M-1/2/fcde1cac16f9310f369939865be981d6

Peri-operative tissue injury triggers the development of Transplant Coronary Artery Disease (TCAD). Animal studies have shown that induction of heme oxygenase (HO)-1 protects the donor organ from the development of TCAD. To investigate the role of HO-1 in TCAD after clinical heart transplantation, we measured intragraft mRNA expression of HO-1, HIF-1[alpha], TGF-[beta], FLIP, and the Bcl-2/Bax balance. Immunohistochemical staining of HO-1 was performed to determine its origin. Myocardial biopsies taken at the end of the transplantation procedure (time 0), at 1 week and at 10 months after transplantation were studied from recipients with or without angiographic signs of accelerated TCAD, diagnosed after 1 year. At time 0, no differences in mRNA expression for any of the measured parameters were found between TCAD positive and negative patients. At 1 week, mRNA expression of HO-1 and TGF-[beta] was higher in grafts that developed accelerated TCAD (p=0.001 and p=0.0002). These higher mRNA levels were accompanied by a pro-apoptotic shift in Bcl-2/Bax (p=0.02), suggesting proneness for apoptosis via the mitochondrial pathway. Immunohistochemical staining showed that HO-1 was mainly produced by infiltrating macrophages. At 10 months, again HO-1 and TGF-[beta] levels were high in TCAD positive patients (p=0.02 and p=0.05), but the expression of apoptotic markers was comparable at this time point. Our results suggest that a higher HO-1 by macrophages in our patient population might be an adaptive response to tissue injury and inflammation, reflecting damage due to the transplantation procedure that finally results in TCAD.


http://www.sciencedirect.com/science/article/B6W6V-46NX397-4/2/949f1e99cfc9fd7fd8c270550649beb4

IL-2 and IFN-[gamma] are associated with acute rejection (AR) and graft vascular disease (GVD) after clinical heart transplantation. Polymorphisms in the genes of IL-2 (T-330G in the promoter) and IFN-[gamma] (CA repeat in the first intron) influence the production levels of these cytokines. Therefore, these polymorphisms might have an effect on the outcome after transplantation. To investigate possible effects of genetic variations in IL-2 and IFN-[gamma] genes on AR and GVD, we analyzed the IL-2 T-330G and the IFN-[gamma] CA repeat polymorphism in DNA of 301 heart transplant recipients. No associations were found for allele or genotype distributions between patients with or without AR (IL-2 allele frequency: P=0.44, genotype distribution: P=0.46; IFN-[gamma] allele frequency P=0.10, genotype distribution 12 repeats allele: P=0.21). Also, no associations were found analyzing the number (0 vs. 1 vs. [ges]1) of AR (IL-2 allele frequency: P=0.59; genotype distribution: P=0.37; IFN-[gamma] allele frequency: P=0.27, genotype distribution 12 repeats allele: P=0.41) or analyzing the polymorphisms in patients with AR within the first month or thereafter (IL-2 allele frequency: P=0.45, genotype distribution: P=0.38; IFN-[gamma] allele frequency: P=0.21, genotype distribution 12 repeats allele: P=0.41). Analyzing both polymorphisms in relation to GVD, resulted in comparable allele and genotype distributions (IL-2 allele frequency: P=0.75; genotype distribution: P=0.77; IFN-[gamma] allele frequency: P=0.70, genotype distribution 12 repeats allele: P=0.63). In conclusion, we did not detect an association between the IL-2 T-330G promoter polymorphism and CA repeat polymorphism in the first intron of the IFN-[gamma] gene and AR or GVD after heart transplantation.

http://www.sciencedirect.com/science/article/B6W6V-436FSJ9-5H/2/054cb171b5951d0376c896011a762401

The rejection of discordant foetal pig islet xenografts in nonimmunosuppressed nonobese diabetic (NOD) mice is dominated by polymorphonuclear cell infiltration whereas allografts are almost exclusively infiltrated by mononuclear cells. To determine if this variation is due to different proinflammatory factors generated at the graft site, we analysed graft-site mRNA expression of various cytokines, and the eosinophil attractant chemokine, eotaxin, in a renal subcapsular islet transplant model using organ cultured foetal pig (xenograft) and foetal BALB/c (allograft) pancreas in prediabetic NOD mice. Using semiquantitative RT-PCR on samples recovered at multiple time points during the first 15 post-transplantation days from mice transplanted with either allogeneic or xenogeneic tissue, we found increased expression of IL-2, IL-4, TNF-[beta] and IL-10 mRNAs at the peak of the cellular infiltrate (on day 5) in both xenografts and allografts but, in contrast to the allografts, no enhanced transcription of IFN-[gamma] mRNA in the rejecting xenografts. When an allograft and a xenograft were placed at the opposite pole of the same kidney the histological appearance of the rejecting allograft site resembled the xenograft site with significant numbers of eosinophils in both, and enhanced expression of eotaxin and iNOS. Additionally, the xenograft response, unlike the allograft response, was marked by an early increased expression of TNF-[alpha] and IL-S (day 3) and an almost complete absence of IFN-[gamma] expression. The results suggest a distinct cell-mediated mechanism for rejection of local pancreas xenografts compared to the rejection of foetal pancreas allografts.


http://www.sciencedirect.com/science/article/B6W6V-45TY3YH-H/2/dbdd2db806a1469824b30238183bf8ac

In this review, we describe the platelet surface molecules with special focus on the polymorphic glycoproteins giving rise to the human platelet alloantigen (HPA) system. We list the platelet glycoprotein complexes GPIa/IIa, GPIb/IIIa, GPIb/IX and some other molecules, the corresponding genes that encode them and we describe their polymorphisms as well as their physiological function. Based on data obtained by serological and molecular methods, we explain how ancestral HPA alloepitopes have developed into the modern variants. We also describe the tissue distribution of these proteins, which seems to be wider than thought initially, and discuss the significance of the HPA encoding genes distribution in various populations. Methods for their determination are then described briefly. Since HPA alloepitopes can induce antibodies with resulting clinical conditions such as: post-transfusion refractoriness to platelets (PTR); post-transfusion thrombocytopenic purpura (PTTP); and fetomaternal alloimmune thrombocytopenia (FMAIT), the mechanism of this alloimmunization and its prevention is described. Although the humoral arm is more important from the clinical viewpoint, the activation of the cytotoxic arm by HPA alloepitopes is also possible. These polymorphisms also seem to have a role in certain other clinical circumstances, therefore their impact on cardiovascular diseases and haemostatic disorders as well as their role in the transplantation of solid organs and bone marrow is addressed. We conclude that during the last decades, the research of the platelet membrane molecules contributed considerably to the diagnostics, prevention and therapy of the blood coagulation and haemostatic disorders, to the management of the neonatal thrombocytopenias, transfusion-related thrombocytopenias, refractoriness to platelet transfusions and autoimmune disorders. It also changed our view on the role of HPA alloepitopes in bone marrow and solid organ transplantation. In the future, this accumulated knowledge will be useful for the development of the cell-based therapies and immune modulation of both acquired and hereditary
diseases.


http://www.sciencedirect.com/science/article/B6W6V-4645DB6-1/2/703ae7a612897d63ca7266e6102b278

Objectives: The severe combined immune deficient (SCID) mouse provides a neutral environment to study human immune responses. We therefore tested human gene expression of Interleukin (IL) 2, 4 and 10, interferon gamma (IFN[gamma]); transforming growth factor beta 1 (TGF[beta]1); and CD40 ligand (CD40L) in splenic extracts of SCID mice after engraftment of PBLs from two persons (direct MLR) or one person plus allopeptides (indirect MLR) in the presence or absence of cyclosporin A (CsA) or FK506. Methods: Cytokine gene expression was detected by RT and quantitative (for IFN[gamma], TGF[beta]1 and CD40L) PCR. All cells, allopeptides, CsA (25 mg/kg/day for 7 days) or FK 506 (0.5 mg/kg/day for 7 days) were administered intraperitoneally (IP). Results: In both direct and indirect MLR the numbers of SCID mice expressing the human cytokine genes varied between 33% for IL4 and 100% for IL10, IFN[gamma], TGF[beta]1, and CD40L. There was significant interpersonal variation in levels of gene expression. Concomitant CsA or FK506 administration for 7 days did not abrogate early or late (1 week after discontinuation of CsA or FK506) cytokine gene expression in either the direct or indirect MLR, but paradoxically enhanced levels of IFN[gamma], TGF[beta]1 and CD40L gene expression in some experiments. Conclusions: The results explain late rejection after rapid calcineurin inhibitor withdrawal or reduction, and illustrate the potential use of SCID mice as a surrogate model to study graft outcome by determination levels of gene expression and sensitivity to immunosuppressive agents in the in vivo alloresponse.


http://www.sciencedirect.com/science/article/B6W6V-42JHD55-1/2/8cd26458bf2a03d407e1dde729b29529

CD40 ligand (CD40L) is important for T/B lymphocyte interaction. To understand the cellular basis of humoral allosensitization we, therefore: (1) measured CD40L protein and gene expression in sensitized and non-sensitized uremic unactivated peripheral CD4+ T lymphocytes; (2) studied the impact of blocking the CD40/CD40L pathway on alloreactive antibody (allo-Ab) production by engrafted sensitized PBLs into severe combined immunodeficient (SCID) mice after in vitro preactivation with IL2/LPs/HLA class II allopeptides and adjuvants as a potent stimulus to produce allo-Ab (Shoker et al. Transplantation 1999;68;1188); and (3) studied the modifying effect of CD40/CD40L blockade on T helper type I and II cytokine gene expression in the respective mice spleen. The CD40L protein was measured by flow cytometry and the gene expression was measured by quantitative RT-PCR. Alloreactive antibodies (alo-Abs) produced by sensitized PBLs engrafted into SCID mice with and without blockade of the CD40 receptor were measured by the PRA-STAT ELISA method. The modifying effects of CD40 blocking on allo-Ab production and cytokine gene expression by the engrafted cells measured by RT-PCR were then compared. There was no detectable CD40L protein expression in the uremic or the control groups. The CD40L gene expression of 0.044+/-.02 attomoles (aM) in the sensitized group was significantly higher than in the non-sensitized patients (0.009+/-.007 aM, P+ T cells (0.016+/-.004 aM, Pn=10); decreased the mean+/-.S.D. optic density of allo-Ab to 0.1+/-.0.13 and the mean+/-.S.D. PRA to 12+/-.16). In the presence of the control Ab, allo-Ab production in SCID sera was present in 100% of the 10 SCID mice tested; the mean+/-.S.D. PRA was 75+/-.20, and the
mean +/- S.D. OD activity was 0.412 +/- 0.17. All cytokine genes were, otherwise, expressed in the presence or absence of CD40 blockade. The results suggest a potential role of an enhanced CD40/CD40L interaction in the sustenance of alloreactive antibody production without significant deviation to T helper-like I or II responses. Blocking the CD40/CD40L pathway may have a potential therapeutic benefit to treat sensitized uremic patients.


http://www.sciencedirect.com/science/article/B6W6V-42JHD55-8/2/bfde831c17749443cabb058edbe1b

Although some previous studies have indicated the possibility of immunosuppression withdrawal in clinical liver transplantation, the mechanism of graft acceptance is not clear. The aim of this study is to elucidate the alloreactivity against the donor and intragraft cytokine profiles in living donor liver transplant (LDLT) recipients with graft acceptance. In October 1999, we had 23 patients who survived without immunosuppression after LDLT with a median drug-free period of 25 months (range: 3-69 months). They consisted of six patients who were electively weaned by an elective weaning protocol and 17 either forcibly or accidentally weaned patients due to various causes but mainly due to infection. We evaluated the alloreactivity against the donor in these patients by a mixed lymphocyte reaction and intragraft cytokine profiles by real-time reverse transcriptase-polymerase chain reaction. The development of donor-specific hyporeactivity was observed in the patients with graft acceptance. The cytokine pattern in the supernatant of the culture medium revealed a down regulation of T helper (Th) 1 cytokine INF [gamma] against the donor while no significant difference was seen in Th2 cytokine IL-10. Regarding the intragraft cytokine profiles, we could find no amplification of Th1 cytokines (IL-2, INF [gamma]) and IL-4 while some of the patients revealed a gene expression of IL-10 with no significant difference from that of the normal, untransplanted liver specimen. In addition, no difference was observed in any other cytokines (IL-1[beta], IL-8, IL-15, TNF[alpha]) compared with those of the normal controls. We propose that the down regulation of Th1 cytokine is one possible mechanism of graft acceptance in LDLT recipients.


http://www.sciencedirect.com/science/article/B6W6V-436FS91-46/2/36c87014cc584b1abb38c428b08286f3

Cytomegalovirus (CMV) infection represents a significant morbidity factor for transplant recipients. CMV infection has an association with the development of allograft rejection (AR) through graft endothelial cell (EC) damage, but the mechanisms are not yet clear. There are few reports addressing the role of humoral immunity in vascular EC injury mediated CMV infection whereas many reports are available regarding the mechanism(s) of CMV-associated allograft EC injury mediated by cellular immunity. Here we examine the incidence of CMV infection in 40 cardiac and 25 renal allograft recipients using polymerase chain reaction (PCR) techniques. We also monitored sera for the development of anti-EC antibodies (AECA0 using an ELISA with human umbilical vein ECs as targets, and IL-2 levels using an ELISA. AECA levels (immunoglobulin-G and immunoglobulin-M) were significantly elevated in allograft recipients who demonstrated CMV-PCR positivity when compared with the CMV-PCR negative group (IgG: 23.1 +/- 16.4 vs 4.7 +/- 4.5, p [tau]0.0001; IgM: 47.0 +/- 53.6 vs. 7.0 +/- 11.2, p[tau]0.0001). Serum AECA (IgC and IgM) levels increased one to four weeks after CMV DNA was detected and elevated AECA levels persisted for at least one to two months, and sometimes for several months. Elevated AECA levels...
correlated well with serum IL-2 levels. These results suggest that CMV infection is associated with an increased humoral immune response to EC antigens, which may be a risk factor for vascular rejection, chronic rejection and decreased allograft survival.


http://www.sciencedirect.com/science/article/B6W6V-436FRKV-P/2/5a44a204fa635f4a4e354fd46408d199

Cytokine gene expression is a critical component of the lung allograft rejection (AR) response and tolerance development in rat models. In order to determine the specificity of cytokine gene expression for AR and tolerance, we examined cytokine (interleukin-2) (IL-2), (gamma-interferon) ([gamma]-IFN), IL-4, IL-10 and tumor necrosis factor-[alpha] (TNF-[alpha]) and control (cyclophilin) mRNA levels in two models of rat lung allograft rejection by RT-PCR (reverse transcriptase polymerase chain reaction), Southern blotting. The first model (WKY->F344) develops a mild to moderate lymphocytic infiltrate on days 14-21 post-transplant (stage II-III AR), which spontaneously resolves by day 35 post-transplant with subsequent development of allograft tolerance (grafts surviving without evidence of AR for> 140 days). Conversely, F344->WKY develops a similar lymphocytic infiltrate by day 14 post-transplant, but by day 21 post-transplant the graft shows severe AR (stage III-IV) and has haemorrhagic infarction with alveolar haemorrhage.

Methods: RNA was extracted from allografts removed on days 3, 7, 13, 21, 35 and 42 post-transplant. Five animals for each group (WKY->F344) and F344->WKY) were examined at each time point, except that no animals in the F344->WKY were examined on day 42. cDNA was synthesized from total extracted RNA and primers specific for rat TNF-[alpha], rat IL-2, rat [gamma]-IFN, rat IL-4, rat-IL-10 and rat cyclophilin were used for gene-specific amplification. (TNF-[alpha], [gamma]-IFN, IL-10, 20 cycles; IL-2, IL-4, 30 cycles; cyclophilin, 20 cycles). The cycles numbers chosen for comparison were found to be optimal during preliminary experiments and occurred during the exponential phase of amplification. PCR products were electrophoresed on a polyacrylamide gel and silver-stained. Gels were subsequently electrotransferred to nylon membranes which were probed with murine cDNAs specific for IL-2, [gamma]-IFN IL-4, IL-10 and TNF-[gamma]. Results: Cyclophilin gene expression was similar for both models at all time points tested; this also served as an internal standard for RT-PCR. In the WKY->F344 tolerance model, TNF-[alpha] mRNA levels were not detectable on days 3 and 7 post-transplant, were at very low levels on day 14 and were undetectable on day 21 post-transplant. In marked contrast, the F344->WKY rejection model showed TNF-[alpha] mRNA present on day 3 which increased markedly on day 7 and peaked on day 14 post-transplant. TNF-[alpha] mRNA levels decreased on days 21 and 35 post-transplant, a time when the lung was undergoing AR. The pattern of IL-2 and [gamma]-IFN mRNA expression was similar to that for TNF-[alpha]. However, IL-2 mRNA was clearly detectable in the WKY->F344 tolerance model on day 7 and [gamma]-IFN was not present until day 14 post-transplant. The F344->WKY rejection model showed very high levels of IL-2 and [gamma]-IFN on day 3 which peaked on day 14. The ratio of IL-2/IL 10 in the F344->WKY rejection model was more than five times that seen in the WKY->F344 tolerance model on day 3 (p WKY rejection model than in the WKY->F344 tolerance model (p WKY rejection model than in the rejection model (p Conclusions: 1) The WKY->F344 tolerance model develops mild to moderate lymphocytic infiltrates on day 14 which is associated with low level IL-2, [gamma]-IFN and TNF-[alpha] gene expression. IL-10 and IL-4 are present at day 3; however, by day 14, IL-10 is the predominantly expressed Th2 cytokine and IL-4 is not expressed. The infiltrates ultimately resolve and the animals develop a functional tolerance to their grafts. 2) The F344->WKY rejection model shows similar lymphocytic infiltrates on day 14, but diverges to a severe rejection which is associated with very early and high levels of IL-2, IL-4, [gamma]-IFN and TNF-[alpha] mRNA expression. IL-10 is not expressed on day 3, and only poorly expressed on day 14. 3) These data suggest that delayed and low level expression of cytokines can be seen in animals developing tolerance t their
grafts while early and abundant expression of cytokines (IL-2, IL-4, [gamma]-IFN and TNF-
[alpha]) is associated with AR and ultimate graft loss. It also appears that, in this system, IL-10
expression is critical for development of tolerance while IL-4 expression occurs early in both the
rejection and tolerant models, but it expressed poorly in the tolerant models on day 14.

Transplantation Proceedings (9)


http://www.sciencedirect.com/science/article/B6VJ0-3W9KWTK-
CD/2/762c7bd20180a9f9a434ccf3f0cc583e


http://www.sciencedirect.com/science/article/B6VJ0-3X2B8S5-
7G/2/a1ff887787c18d45657a04086f495a3


http://www.sciencedirect.com/science/article/B6VJ0-3W9KWTK-
7P/2/6b4f41d4cef57e746d563d8f64e5d4a4

Transplantation Proceedings 33(7-8): 3304.

http://www.sciencedirect.com/science/article/B6VJ0-44N9JF0-
3V/2/be7fc0c09fc1dd548310681548543e67

Lazzereschi, D., M. Forni, et al. (2000). "Efficiency of transgenesis using sperm-mediated gene transfer:

http://www.sciencedirect.com/science/article/B6VJ0-40WDV4D-
13/2/62d4f2fe60a8491e9f0bcd049c5637a7

Nakajima, H. and T. Oka (1997). "Analysis of biochemical and biological functions of Fas-Ligand (FasL)
and fas on activated T cells in allo-immune response." Transplantation Proceedings 29(1-2):

http://www.sciencedirect.com/science/article/B6VJ0-3VN9BV8-1D/2/c4584f01210976fd9f5ef8d90f63f280

The aim of this work was to determine the expression of cyclooxygenases (COX-1 and COX-2) during acute human renal allograft rejection. RT-PCR and immunohistochemistry were performed. The COX-2 mRNA was more abundant than COX-1 mRNA in the group with acute rejection (P = .04). COX-2 protein was more abundant than COX-1 protein in the group with acute rejection, including podocytes (P = .003), and interstitial cells (P < .001). In conclusion, COX-2 which is up-regulated during acute human renal allograft rejection, may play a role in renal inflammation.


http://www.sciencedirect.com/science/article/B6VJ0-4CKJVPX-G/2/ffb82bc6c324ec525600051754e99a9b

Clinical islet transplantation is now established as a treatment for patients with type I diabetes. Although organs from brain-dead (BD) donors are the main source for clinical transplantation, marginal status after BD produces deterioration of the organs followed by molecular activation. The effect of brain-death (BD) induction on the immunological status of donor islets was investigated using a rodent model of BD. BD animals showed decreased levels of peripheral white blood cells (WBC) compared to controls, indicating the extravasation of these cells (7270 +/- 50 vs 9570 +/- 370, respectively). In a densitometric study of RT-PCR products, the Th2 cytokine (IL-10) was significantly up-regulated in BD (2.91 +/- 0.26 vs 1.76 +/- 0.40), but a Th1 cytokine (IL-2) showed minimal change. Increased expression of IL-10 may inhibit macrophage function. As the marginal status after BD deteriorates, the islets of these donors may display early graft loss or poor long-term function. Integrative studies of immunomodulation might be necessary to eliminate islet infiltrates.


http://www.sciencedirect.com/science/article/B6VJ0-475HWXG-33/2/247cb29c3bf5d4a0488d0ea2ed7c0be4

*Trends in Genetics* (2)

http://www.sciencedirect.com/science/article/B6TCY-4C89G52-D/2/4caa610d0886ded94e7e6386a1ba5188


http://www.sciencedirect.com/science/article/B6TCY-47DVCJN-6W/2/7a3fced38382e85a1427fa1c35366ec1

Tubercle and Lung Disease (1)


http://www.sciencedirect.com/science/article/B6WXJ-4D6XP4P-3/2/51098ba49d1f656d04e4cb38a6ea1e0f

Objective: To compare 3 immunoassays, an immune complex assay, and an application of the polymerase chain reaction (PCR) for the diagnosis of tuberculous meningitis (TBM). Material: Cerebrospinal fluid (CSF) from 33 patients with TBM and from 34 control patients with infectious and non-infectious CNS diseases was analysed. Results: The antibody immunoassays were either nonspecific or insensitive. However, detection of mycobacterial IgG immune complexes correlated strongly with infection, as they were detected in the CSF from 64% of the patients with TBM compared to only 3 (9%) of the control samples. PCR analysis, using Mycobacterium tuberculosis-specific oligonucleotide primers, also strongly correlated with infection, as DNA was amplified from 54% of the samples from patients with TBM, but from only 2 (6%) of the control samples. Both 'false positive' samples were also positive in the immune complex assay and came from 2 patients with otogenic brain abscesses. It is conceivable that these patients suffered from otogenic tuberculosis with secondary non-mycobacterial meningitis. When combining the immune complex assay with DNA-detection by PCR, 100% of the culture positive and 74% of culture negative samples were found to be positive, while maintaining a high specificity. Conclusion: Parallel analysis to detect mycobacterial immune complexes and M. tuberculosis-specific DNA by PCR from the CSF of patients may offer a sensitive and specific tool for the diagnosis of TBM.

Tuberculosis (4)

http://www.sciencedirect.com/science/article/B6WXK-4FRKVJR-2/2/9f54e8034d300928d820472abfa51e7c

**Summary**

**Setting:** The ability of chemokines such as macrophage inflammatory protein (MIP)-1[alpha], MIP-1[beta], and regulated-upon-activation, normal T cell expressed and secreted (RANTES), to attract and activate T cells and monocytes, the building blocks of the granuloma, suggests that these chemokines may have a role in modulating immune responses to Mycobacterium tuberculosis infection.

**Objective:** We hypothesized that the chemokine receptor 5 (CCR5) ligands, MIP-1[alpha], MIP-1[beta] and RANTES, are virulence correlates in M. tuberculosis infection and are indispensable to granuloma formation.

**Design:** The ability of virulent (H37Rv) and avirulent (H37Ra) strains of M. tuberculosis to induce chemokine production in vivo and in vitro was determined at protein and mRNA levels. We also compared bacterial burden, and granuloma numbers and size in H37Rv-infected CCR5-/- or wild-type C57BL/6 mice.

**Results:** In vivo, lung mRNA and protein measurements of MIP-1[alpha], MIP-1[beta] and RANTES indicate significantly higher (p values (days 14-28) in the H37Rv-infected than the H37Ra-infected mice. This is consistent with a higher infection burden of the virulent strain. However, in vitro alveolar macrophage stimulation by H37Rv or H37Ra yielded no significant differences in production of the three chemokines at all time points. Histological analysis of granulomas did not show any significant differences in granuloma numbers, size and M. tuberculosis growth in CCR5-/- compared to wild-type mice.

**Conclusions:** The production of the CCR5 ligands, MIP-1[alpha], MIP-1[beta], and RANTES, does not clearly correlate with virulence of M. tuberculosis. These ligands and their receptors may not be indispensable to the development of granulomas in murine tuberculosis.


http://www.sciencedirect.com/science/article/B6WXK-4B0KWVV-3/2/adecf1a017b1c9dcd81b9d54350279d4

The RD1 locus is deleted from all strains of Mycobacterium bovis BCG but present in virulent isolates of M. bovis and Mycobacterium tuberculosis. The RD1 gene Rv3879c encodes a proline-and alanine-rich protein that shows sequence polymorphism across members of the M. tuberculosis complex. The role of this protein in virulence was investigated by deleting the Rv3879c homologue from M. bovis (Mb3909c) and testing the virulence of the mutant in the guinea pig model. The M. bovis [Delta]Mb3909c mutant was not attenuated in the guinea pig model, showing that this gene does not encode a virulence factor and plays no role in the attenuation caused by loss of RD1.


http://www.sciencedirect.com/science/article/B6WXK-4BRB74S-1/2/3eab7d0e08d311f1e55705ddc237f53856

Resistant Mycobacterium tuberculosis has become a serious threat to public health. In order to inhibit spreading and give effective treatment, it is of great importance, as early as possible, to detect drug-resistant bacteria. To evaluate the usefulness of the Line Probe Assay (INNO-LiPATM Rif.TB) for rapid detection of rifampicin resistance, we used 52 clinical isolates of M.
tuberculosis from the national strain collection at the Swedish Institute for Infectious Disease Control and the drug susceptible reference strain H37Rv. By using BACTEC 460 methodology, 27 of these strains were determined as resistant to rifampicin and 26 as sensitive to rifampicin. Mutations known to give resistance to rifampicin were detected by LiPA in all 27 rifampicin-resistant strains. Among the 26 susceptible strains, 24 had the wild-type pattern in LiPA, while in two, mutations were seen. The LiPA correctly identified the M. tuberculosis complex in all samples. The high accuracy and simplicity of LiPA makes it a very promising method for the early identification of rifampicin resistance in M. tuberculosis.

Wedlock, D. N., M. A. Skinner, et al. (2003). "Vaccination with DNA vaccines encoding MPB70 or MPB83 or a MPB70 DNA prime-protein boost does not protect cattle against bovine tuberculosis." Tuberculosis 83(6): 339.

http://www.sciencedirect.com/science/article/B6WXK-49FGP7B-2/2/85fb80117c918d3c07af2e7b6614493e

Setting: Bovine tuberculosis is a problem in a number of countries and protection of cattle by vaccination could be an important control strategy. Objectives: To determine the ability of DNA vaccines, which express the mycobacterial antigens MPB83 and MPB70 and a DNA prime-protein boost strategy to stimulate immune responses in cattle and protect against bovine tuberculosis. Design: Groups of cattle (n=10) were vaccinated with MPB83 DNA, MPB70 DNA, or MPB70 DNA followed by MPB70 protein or injected with BCG or control plasmid DNA. Animals were challenged intratracheally with virulent Mycobacterium bovis at 13 weeks and protection assessed 17 weeks later at postmortem. Results: In contrast to the strong cellular immune responses induced by BCG, the DNA vaccines induced minimal interferon-gamma (IFN-[gamma]) and interleukin-2 (IL-2) responses. Cattle primed with MPB70 DNA and boosted with MPB70 protein induced a strong antibody response and a weak IFN-[gamma] response. BCG gave significant reduction in four pathological parameters of disease while the DNA vaccines and MPB70 DNA/protein did not protect animals against challenge with M. bovis. Moreover, cattle vaccinated with MPB70 DNA/protein had a significantly higher proportion of animals with severe lung lesions (>100 lesions) than the MPB70 DNA alone or the control group. Increased bovine PPD-specific IL-4 mRNA expression in cattle, post-challenge, correlated with the presence of tuberculous lung lesions. Conclusion: Vaccination of calves with MPB70 or MPB83 DNA vaccines or with a more immunogenic MPB70 DNA prime-protein boost strategy did not induce protection against bovine tuberculosis.

Ultrasound in Medicine & Biology  (1)


http://www.sciencedirect.com/science/article/B6TD2-4B3N204-F/2/1257b33945bdacd1048b3a717c23ede1

This study was designed to examine the effects of pulsed low-intensity ultrasound (PLIUS) on chondrocyte viability, proliferation, matrix production and gene expression. Chondrocytes were
isolated from the distal part of the sternum of 16-day-old chick embryos and cultured in alginate beads. PLIUS at 2 mW/cm² (group PLIUS2) and 30 mW/cm² (group PLIUS30) was applied to chondrocytes for a single 20-min treatment. A control group was treated without PLIUS. The viability of chondrocytes was not affected by exposure to PLIUS. PLIUS influenced chondrocyte proliferation in an intensity-dependent manner. By day 7 after application of PLIUS, the gene expression and synthesis of aggrecan was the same as in the controls. At this same time point, the expression and synthesis of type II collagen was not different between the controls and PLIUS30, but was increased in PLIUS2. PLIUS was shown to inhibit the expression of type X collagen. This inhibition of chondrocyte hypertrophy may prove to be significant in the management of cartilage degeneration. (E-mail: spencer@helix.nih.gov)

**Urologic Oncology** (4)


http://www.sciencedirect.com/science/article/B6TD3-45BCDY7-4/2/2d7d14673c3aea4196579a7ec67b8ca4

The function of p21 is related to cell apoptosis, progression and malignancies. It is thought that p21 is related to cancer formation but is not related to tumor grade. We aimed to investigate the polymorphism of p21 codon 31 as a candidate for the genetic marker of bladder cancer and its progression. The distribution was analyzed in 53 bladder cancer patients, 119 healthy controls in Taiwanese patients. Polymerase chain reaction based restriction analysis was used for the study of the association of p21 codon31 polymorphism with bladder cancer. There was a significant difference in p21 codon 31 polymorphism between the control and the cancer patients (p<0.05). Serine heterozygote was more prominent in the invasive group with 25 to 1% respectively when compared with the non-invasive group. The polymorphism of p21 codon 31 is associated with bladder cancer. An individual possessing one allele of arginine form in p21 codon 31 has a higher risk of developing bladder cancer than the serine form. Although the mechanism is unclear, our results show p21 gene is associated with tumor grade.


http://www.sciencedirect.com/science/article/B6TD3-3XWJR6F-4/2/63ae78d8fad5d388b6f344d7444dca85c

The transforming growth factor-[beta] (TGF-[beta]) signal transduction system has been reported to play a role in prostate tumorigenesis and the regulation of cell cycle-related gene expression including the cyclins, cyclin dependent kinases (Cdks), and Cdk inhibitors. The objective of this investigation was to examine the expression of TGF-[beta] receptors I and II and five cell cycle-related genes--Cdk-4, p15, p21(WAF1/CIP1), p27, and cyclin E--in three prostate carcinoma cell lines and normal prostate by quantitative reverse transcriptase (RT)/polymerase chain reaction (PCR). The expression of the TGF-[beta] receptor II was reduced by 5.5- and 2.2-fold in the LNCaP and DU145 cells, respectively, compared with normal prostate tissue. A similar decrease was observed for the TGF-[beta] receptor I transcript in the LNCaP cells. In addition, 20-fold less
The expression of specific cell cycle-related genes may be entirely or partially regulated by alterations in TGF-[beta] pathway and may play a role in prostate carcinoma development.

http://www.sciencedirect.com/science/article/B6TD3-438KG8V-6/2/d51b3c09dae45f3b7e0a015aae9093fe

Circulating soluble Fas (sFas) and expression of Fas-ligand on cancer cells are mechanisms of immune escape. The aim of the present study was to investigate expression and production of Fas and Fas-ligand on bladder cancer cell lines of different grade as a basic mechanism of their secretion in vivo. sFas and sFas-ligand serum levels of patients with different stage of bladder cancer were examined to determine the possible clinical use of these molecules as tumor markers. Bladder cancer cell lines RT4 (G1), RT112 (G1), T24 (G3) and SUP (G4) were analyzed by flowcytometry for Fas and Fas-ligand expression. To determine if the Fas-ligand gene is transcribed in these bladder cancer cell lines, RT-PCR was performed on mRNA extracted from these cell lines. Production of sFas and sFas-ligand was examined in cell culture supernatants of
the cancer cells as well as in the serum of 62 patients with bladder cancer by a specific ELISA test. We demonstrate that Fas is expressed in similar levels on all human bladder carcinoma cell lines. In T24 (G3) and SUP (G4) cell lines we were able to detect the Fas-ligand protein, whereas no Fas-ligand protein could be found in RT4 and RT112 (G1) cells. Fas-ligand mRNA was expressed in all bladder cancer cell lines. Furthermore, all bladder cancer cell lines produce sFas but no sFas-ligand in spite of mRNA expression. The range of sFas levels in the serum of all patients with bladder cancer was large and did not show a correlation to the histopathological stage of bladder cancer. Although there is in vitro evidence that sFas and Fas-ligand play a role in bladder cancer, no correlation between the sFas and sFas-ligand serum levels and the histopathological stage of bladder cancer could be found. Therefore, serum sFas and sFas-ligand have to date limited clinical relevance.

**Urologic Oncology: Seminars and Original Investigations (5)**


http://www.sciencedirect.com/science/article/B6TD3-3Y45XM7-2/2/afb5e9e74e38bc17a38ed76ec601dc36

The present study was designed to investigate the mRNA expression of four DNA repair genes (XPCC, hMSH2, XRCC 1, and ERCC 1) in human fetal and adult prostatic tissues and cancer cell lines using differential reverse transcriptase-polymerase chain reaction (RT-PCR). For this purpose, total RNA from four human prostate cancer cell lines (LNCaP, PC-3, DU-145, and ND-I) and human fetal (n = 10) and adult (n = 10) prostates was extracted and mRNA expression was analyzed for four DNA repair genes by RT-PCR using specific oligonucleotides. For quantitation, we used [beta]-actin as an internal standard in each tube as baseline gene expression. The results of these experiments suggest that human prostate cancer cell lines (LNCaP, PC-3, DU-145, and ND-I) have 2- to 10-fold lower mRNA expression for all four DNA repair genes compared with human fetal and adult prostatic tissues. Expression of DNA repair gene XPCC was about 10- to 15-fold lower in prostate cancer cells compared with fetal and adult prostatic tissues. This study, for the first time, demonstrates that mRNA levels of DNA repair genes in human prostate cancer cells are significantly lower compared with the adult prostate.


http://www.sciencedirect.com/science/article/B6TD3-49D1VPF-5/2/653ff1e959227082e653bd84ec0a5f24

Prostate cancer is the most common urological malignancy in Taiwan. The formation of prostate cancer has been reported to be associated with androgen. Two key steps in the sex steroid synthesis are mediated by the enzyme cytochrome P450c17[alpha] which is encoded in the CYP17 gene. Our aim was to investigate whether a polymorphism of CYP17 gene could be used as a genetic marker for associating prostate cancer. In this study, we compared the frequency of the C/T polymorphism of CYP17 gene 5'-UTR promoter region between 93 patients with prostate cancer and 121 healthy male volunteers (age, >60 years). The result revealed no significant
association between the CYP17 genotype and prostate cancer ($P = .781$). Therefore, CYP17 C/T polymorphism is not a valid genetic marker for prostate cancer. Although a possible interaction between CYP17 gene C/T polymorphism and SP-1 transcription factor has been reported in the literature, we did not find any evidence for this the difference among clinical staging, pathological grading, or responsiveness to hormonal therapy in prostate cancer.


http://www.sciencedirect.com/science/article/B6T3D-4F26RJ6-6/2/aa07e348a5ae24bf4f00baaf41bd62a8c

The purpose of the present study is to investigate the role of X-linked inhibitor of apoptosis protein (XIAP) in the regulation of apoptosis induced by cisplatin in human prostate cancer cell line (LNCaP). We examined the effects of cisplatin on cell growth and apoptosis in LNCaP by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1), flow cytometric analyses, and caspase-3 activity assay. In addition, to clarify the roles of the XIAP, we established clonal cell lines that overexpressed XIAP. The effects of cisplatin on the XIAP expression in the induction of apoptosis in LNCaP were examined by RT-PCR and immunoblot analyses. Although the growth rates were reduced in a dose- and time-dependent manner by cisplatin in LNCaP sublines, the anti-proliferative effects of cisplatin were significantly decreased in XIAP stably overexpressing cell lines. In addition, we found that cisplatin-induced apoptosis following activation of caspase-3, and that the overexpression of XIAP inhibited apoptosis by attenuating caspase-3 activity. Interestingly, treatment of LNCaP cells with 10 and 100 μM cisplatin for 48 h significantly decreased the expression of XIAP at both the protein and mRNA levels in a dose-dependent manner. Furthermore, 10-μM cisplatin treatment of LNCaP decreased XIAP mRNA and protein in a time-dependent manner. These results suggest that cisplatin induces apoptosis by the inhibition of XIAP expression, and that XIAP plays an important role in the regulation of cisplatin-induced apoptosis in LNCaP cells. The ability of cisplatin to down-regulate XIAP may be an important mechanism in chemosensitivity.


http://www.sciencedirect.com/science/article/B6TD3-3XSK186-J/2/b3bebdd1111509c488d01d50a9742e3b

It has yet to be determined whether the detection of prostate specific antigen (PSA)-expressing or prostate specific membrane antigen (PSM)-expressing cells in the circulation of prostate cancer patients is a more accurate predictor of clinical outcome. A method of evaluating both markers simultaneously would aid in the determination of the clinical relevance of reverse transcriptase polymerase chain reaction (RT-PCR) as a staging tool for prostate cancer. We describe the development of a multiplex RT-PCR assay that simultaneously detects the presence of both PSA-expressing cells and PSM-expressing cells, as well as a ubiquitously expressed internal control all within a single reaction. Both PSA cDNA and PSM cDNA were concurrently amplified by multiplex PCR using LNCaP mRNA as the starting template. When used as part of a nested PCR system, the multiplex RT-PCR assay identified one prostate cancer cell when placed in a background of one million cultured B lymphocytes. The multiplex assay was then applied to mRNA isolated from metastatic prostate cancer patients and from healthy male and female
volunteers. While all were positive for the internal control G3PDH, three of seven prostate cancer patients were positive for both PSA and PSM expression and two more were positive for either PSA or PSM. None of the male or female volunteers were positive for either PSA or PSM. Multiplex RT-PCR allows for the amplification of both PSA and PSM cDNA within a single RT-PCR reaction, and this approach should allow a consistent comparison of the clinical utility of both PSA and PSM markers as staging tools and predictors of response to therapy.


http://www.sciencedirect.com/science/article/B6TD3-3XY2J8K-H/2/08257c8be9de6c487878b53c9518fa1e

The basis for malignancy is the deregulation of the cell cycle resulting in uncontrolled cellular proliferation. This review examines our current understanding of peptide growth factors, stimulatory and inhibitory signaling cascades, protooncogenes and tumor suppressor genes, cyclin dependent kinases and cyclins as they relate to the cell cycle. The epidermal growth factor and transforming growth factor [beta] transduction pathways are presented to illustrate the complex interactions required for normal signaling and control of the cell cycle. The second part of the review explores the relevance of these pathways and their alterations in the genitourinary malignancies of the prostate, bladder, kidney and testis.

Urology (11)


http://www.sciencedirect.com/science/article/B6VJW-3WDDGNS-1M/2/3ac7f15b8bd045c70076caecde7492dd

Objectives. Cryoablation of the prostate has been reported to induce impotence as a consequence of cavernosal nerve injury. This study is designed to investigate the early and late effects of cavernosal nerve cryoablation on growth factor expression and erectile function in a rat model.

Methods. Forty male rats were divided into two groups (n = 20 each). The first group underwent unilateral cavernosal nerve freezing (experimental group). Before their euthanization at 1 and 3 months (10 rats each), erectile function was assessed by electrostimulation of the cavernous nerves. The second group served as the control and was killed at the same time points. Western blot and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques were used to identify protein and gene expression of nerve growth factor (NGF), transforming growth factor-alpha (TGF-alpha), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1) in the rat penis and pelvic ganglia. Results. Electrostimulation of the frozen nerve after 3 months revealed a significantly higher maximal intracavernosal pressure and a shorter latency period than in the 1-month group. At 3 months, immunoblot showed upregulation of NGF, TGF-alpha, and the precursor form of IGF-1 protein expression in the penile tissue; RT-PCR showed downregulation of NGF gene expression in the pelvic ganglia of the frozen side. Conclusions. The results show that erectile function decreased at 1 month and then partially recovered 3 months after cavernosal nerve freezing. This alteration in erectile function was associated with differential
Gene and protein expression of the growth factors (NGF, TGF-alpha, EGF, and IGF-1). Further studies are required to elucidate the potential role of these growth factors in the prevention and treatment of cryoablation-induced impotence.


http://www.sciencedirect.com/science/article/B6VJW-4599BFT-1R/2/d6d23f7d131e3b705c3a6683ed9014f5

Objectives. To investigate the expression of androgen receptor (AR) coactivators in the human prostate for a better understanding of androgen action in prostate cancer. Methods. Using reverse transcriptase-polymerase chain reaction, we examined the expression levels of AR coactivators (ARA55, SRC1, ARA54, TIF2, RAC3) in four prostate cancer cell lines (DU145, PC3, LNCaP, and LN-TR2), nine benign prostatic tissue samples, and 21 prostate cancer tissue specimens. Results. In the cell lines, SRC1 was expressed ubiquitously at almost equal amounts. Contrary to this, ARA55, ARA54, TIF2, and RAC3 displayed cell line-specific expression. In the LN-TR2 cells, established from LNCaP cells by long-term treatment with tumor necrosis factor-alpha, the expression levels of ARA55 and TIF2 were much higher than those in the LNCaP cells. In every prostatic tissue specimen, the expression levels of TIF2 and RAC3 were very low. The expression levels of ARA55 and SRC1 were higher in the cancer specimens with a higher grade or poor response to endocrine therapy than in those with a lower grade or good response to endocrine therapy. Conclusions. Prostate cancer cells express AR coactivators. Long-term stimulation by tumor necrosis factor-alpha could increase ARA55 and TIF2 expression in LNCaP cells. The different expression of coactivators may contribute to the different response of prostate cancer to androgenic stimulation or endocrine therapy.


http://www.sciencedirect.com/science/article/B6VJW-4BP0PD8-21/2/34f6cb2301034d625abb7f63b8897502

Objectives. To evaluate the utility of methylation-specific polymerase chain reaction analysis of the pi-class glutathione-S-transferase (GSTP1) gene promoter in prostatic secretions for cancer detection and prognostication. Methods. Prostatic secretions were obtained from a total of 100 radical prostatectomy specimens immediately after surgical extirpation. GSTP1 promoter methylation was assessed by methylation-specific polymerase chain reaction analysis using two different primer sets. Correlations between GSTP1 promoter methylation and clinical and pathologic variables were examined. Results. The sensitivity for detection of GSTP1 methylation in prostatic secretions from men with clinically localized prostate cancer using two different primer sets was 76% and 54%. Methylation of the GSTP1 promoter was detected by both primer sets in 44% and by at least one primer set in 86% of the prostatic secretion specimens. The degree of methylation detected in the prostatic secretions was associated with the extent of cancer (predominant involvement of one or both sides of the gland; P = 0.02) and increasing age (P = 0.009). Conclusions. Genomic DNA with GSTP1 promoter methylation can be detected in prostatic secretion specimens from the great majority of men with localized prostate cancer. Assays of GSTP1 promoter methylation in prostatic massage fluid or ejaculate may therefore serve as useful adjuncts to existing methods for prostate cancer screening and prognostication.

http://www.sciencedirect.com/science/article/B6VJW-3VXRW2V-8/2/3d1d7018f61031c0f19ea03ff430c4da

Objectives. A previously reported study using nested polymerase chain reaction (PCR) analysis indicated the presence of DNA from a variety of prokaryotic microorganisms in 77% of transperineal prostate biopsies from patients with chronic nonbacterial prostatitis. Because that study did not include a control group, we investigated whether microbial DNA could also be found in transperineal prostate biopsies obtained from men who did not have a history of prostatitis. Methods. Transperineal biopsies of both lobes of the prostate were obtained under ultrasound guidance from 9 patients with localized adenocarcinoma of the prostate. DNA was extracted from the prostatic tissue and two-round amplification performed using nested primers from a highly conserved region of the bacterial 16s rRNA gene. Amplified DNA was purified and sequenced, and sequences obtained were compared to bacterial rRNA genes recorded in GenBank. Results. Eleven of 18 biopsy specimens from 8 of 9 patients were positive for bacterial DNA by PCR. Sequence data indicated a predominant organism in 8 of 11 specimens, with greater than 95% homology to DNA from several different genera of bacteria, including *Escherichia* and *Bacteroides*. All 9 control samples from the instruments before biopsy were negative. Conclusions. The presence of bacterial 16s rRNA genes in prostatic tissue is not specific for chronic prostatitis and occurred in most of our patients with localized prostate cancer. Whether the presence of such bacteria is related to the development of prostatic diseases such as prostatitis or prostatic cancer will require carefully controlled trials, including appropriate control groups examined identically.


http://www.sciencedirect.com/science/article/B6VJW-494YTJB-1T/2/9d8f6639a9a445f78233425a59474cba

Objectives. To use the Bst U I polymorphism as a genetic marker in the search for the association between patients with prostate cancer and normal control subjects. The formation or progression of prostate cancer is presumed to be associated with a polymorphism of the vascular endothelial growth factor (VEGF) gene. The most frequently seen polymorphism is Bst U I (C to T) located at the -460th nucleotide upstream of the VEGF gene. Methods. A normal control group of 119 healthy people and 96 patients with prostate cancer were examined. The polymorphism was seen after polymerase chain reaction-based restriction analysis. Results. The analysis revealed significant differences between normal individuals and patients with cancer (P = 0.110, Fisher's exact test). Conclusions. The Bst U I polymorphism of the VEGF gene is a suitable genetic marker of prostate cancer but cannot be used in the prediction of the outcome of patients who have received hormonal therapy.


http://www.sciencedirect.com/science/article/B6VJW-4BHSXTW-22/2/2038a918747430cf9837ce10240bca26

Objectives. To investigate the expression of endothelin-1 (ET-1) and adrenomedullin (ADM) in the
renal pelvis, stenotic ureteropelvic junction, and ureter of 20 male Wistar rats with congenital unilateral ureteropelvic junction obstruction; the normal contralateral kidneys served as controls. The molecular pathophysiology of congenital ureteropelvic junction obstruction is still unclear. The implication of altered peptidergic innervation is under discussion. Our study group has recently been able to demonstrate a significant increase in ET-1 and a significant decrease in ADM in prestenotic and stenotic tissue, but not in the remainder of the ureter, compared with controls.

Methods Twenty animals were killed, and samples of the renal pelvis, ureteropelvic junction, upper ureter, middle part of the ureter, and lower ureter were immediately snap-frozen and stored in liquid nitrogen. Total RNA was extracted, and subsequently 1 [mu]g of RNA was reversely transcribed. mRNA expression of ET-1 and ADM was determined semiquantitatively using on-line polymerase chain reaction. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to relate the specific mRNA expression to the expression of a housekeeping gene.

Results We found a significant increase in the expression of ET-1 in the obstructed junctions related to GAPDH (P Conclusions Alterations in the local production of peptidergic neurotransmitters, especially ET-1, may contribute to the molecular pathogenesis of ureteropelvic junction obstruction. Results previously obtained in the stenotic tissue from children were confirmed in the stenotic tissue from the rat model. We hypothesize that the alterations are disease-, but not age-specific.


http://www.sciencedirect.com/science/article/B6VJW-3W847WP-J/2/8c4f52e112274c9f71fc67e1cf9288f3

Objectives. To determine if circulating prostate cells are detectable subsequent to transrectal ultrasound (TRUS)-guided biopsy, and if so, whether cells remain in circulation for up to 4 weeks.

Methods. Blood samples were drawn from 90 patients with elevated serum prostate-specific antigen (PSA) levels and/or abnormal digital rectal examination. Two samples were drawn from all patients immediately prior to TRUS and 30 minutes postbiopsy. Blood samples were also obtained 1 week postbiopsy from 83 patients, and 1 month postbiopsy from 61 patients. Multiplex nested reverse transcription polymerase chain reaction assay (RT-PCR) for PSA and prostate-specific membrane antigen (PSM) was performed on total ribonucleic acid (RNA) from each sample. Results were reported as positive if at least one of the targets was detected.

Results. Of 45 patients with biopsy-proven adenocarcinoma, 22 were RT-PCR positive prebiopsy and all remained positive 30 minutes postbiopsy. Of 23 patients with adenocarcinoma who were RT-PCR negative prebiopsy, 5 (22%) converted to positive 30 minutes postbiopsy (P Conclusions Detection of circulating prostate cells subsequent to biopsy occurred in 11 of 55 (20%) previously RT-PCR negative patients, a proportion twice that reported in the literature. We attribute this higher proportion to the simultaneous detection of PSA and PSM mRNA in our multiplex assay. Conversion rates were similar in patients regardless of biopsy result. Testing of serial postbiopsy blood demonstrates clearing of these cells by 4 weeks in most patients.


http://www.sciencedirect.com/science/article/B6VJW-4DKC7JC-1N/2/201c50f9730a2582cc909e412f4e5bdd

Objectives To determine whether nucleophosmin/B23 mRNA expression in bladder carcinoma predicts recurrence, progression, and survival.

Methods Real-time reverse transcriptase-
polymerase chain reaction was performed on 50 fresh cancer specimens. The change in the cycle of threshold (Ct) was the difference in the Ct values derived from the nucleophosmin/B23 gene assayed and the 18S ribosomal RNA control \[\text{Ct (18S) - Ct (nucleophosmin/B23)}\].

Results Fifty patients diagnosed with bladder cancer were followed up postoperatively for a median of 24 months. Overexpression of nucleophosmin/B23 mRNA was observed in 37.1% of patients with Stage pT1 and 73.3% of those with pT2-T4 disease. Nucleophosmin/B23 overexpression was not associated with tumor grade (P = 0.163) but was associated with bladder cancer recurrence (68.2%) and progression (88.9%) when adjusted for the effects of clinical stage. Multivariate analysis revealed that the overall tumor stage and nucleophosmin/B23 mRNA overexpression were important prognostic indicators for bladder carcinoma (P Conclusion Overexpression of nucleophosmin/B23 mRNA was independently associated with bladder cancer recurrence and progression. In patients with muscular invasion disease, overexpression of nucleophosmin/B23 mRNA was associated with the greatest risk of recurrence and progression, suggesting a potential rationale for early definitive therapy in these patients.


http://www.sciencedirect.com/science/article/B6VJW-3V3MWCB-N/2/7bb1cb93ea0586c8bdf9424aaa8a0144

Objectives. Parathyroid hormone-related protein (PTHrP) is a primary factor in the pathogenesis of malignancy-associated hypercalcemia. By alternative splicing, the human PTHrP gene can generate three different species of mRNA that encode three initial translational isoforms of 139, 173, and 141 amino acids. We recently reported that PTHrP was present in normal prostatic neuroendocrine cells and was overexpressed in prostate cancer tissue as demonstrated by immunostaining. This study was undertaken to further clarify the complex expression of PTHrP gene in normal prostate tissue and prostate cancer. Methods. PTHrP mRNA in samples prepared from normal prostate tissue, prostate cancer, and three prostate cancer cell lines, PC3, LNCaP, and DU145 was assessed using Northern hybridization. Expressed PTHrP isoforms were deduced from differential reverse transcription-polymerase chain reaction (RT-PCR) assays with exon-specific primers. Further localization of different species of PTHrP mRNA was performed using nonradioactive in situ hybridization with exon-specific probes on consecutive sections of normal and neoplastic prostate tissue. Results. Northern hybridization showed that the PTHrP expression level was higher in prostate cancer than in normal prostate tissue. All three PTHrP isoforms could be detected in normal prostate tissues and prostate cancer with differential RT-PCR. Further analysis using in situ hybridization with exon-specific probes revealed that all three PTHrP isoforms were present in prostatic neuroendocrine cells and only PTHrP-1-139 isoform could be clearly detected in prostate cancer tissue. Two androgen-insensitive cell lines, PC3 and DU145, derived from a bone metastasis and a brain metastasis, respectively, expressed all three mRNA species encoding for the three isoforms, but DU145 cells expressed less than PC3 cells. Androgen-sensitive LNCaP cells exhibited a low level of expression of mRNA species encoding for PTHrP-1-139 and PTHrP-1-173, and no expression of PTHrP1-141 isoform. Conclusions. All three initial translational isoforms of PTHrP are produced by prostatic neuroendocrine cells. The mature products of PTHrP might exert their effects on other prostatic epithelial cells in a paracrine fashion and also participate in the homeostatic regulation of the ejaculate. In prostate cancer, differential expression of these three isoforms is evident and PTHrP-1-139 isoform is more abundant than the other two forms. These findings are valuable for designing future research studies to further elucidate the biological functions of PTHrP in normal prostatic glands and prostate cancer.

http://www.sciencedirect.com/science/article/B6VJW-3Y51HYK-12/2/06dd4b234b5363126c8803f7775d71d9

Objectives. To determine whether the number of CAG repeats in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. Methods. Using the polymerase chain reaction, the number of CAG repeats was assayed in 41 patients with idiopathic azoospermia and in 48 normozoospermic fertile men. Results. In the control group, the CAG repeat length ranged from 17 to 30 (mean 23.9 +/- 2.9); in the azoospermic group, the CAG repeat length ranged from 20 to 34 (mean 26.5 +/- 3.5). The difference between the two groups was statistically significant (P = 0.0013). None of the men in the control group had a CAG repeat length greater than 31; four of the azoospermic men had 34 CAG repeats. Conclusions. Results suggest that an increase in the number of CAG repeats in the androgen receptor gene to 31 or greater may be associated with the etiology of at least some cases of idiopathic azoospermia.


http://www.sciencedirect.com/science/article/B6VJW-4BP0PD8-20/2/87a12e7f99967fcb59c54655ae5c63ac

Objectives To explore the expression patterns and possible involvement of leptin and its receptor in the pathogenesis of urinary bladder cancer, with a focus on transitional cell carcinoma. Methods Using reverse transcription-polymerase chain reaction, immunoblotting, and immunohistochemistry techniques, we correlated the expression patterns of leptin and its receptor with the occurrence of transitional cell carcinoma. We also applied transient transfection followed by BrdU labeling and immunofluorescent staining to address the effect of the leptin receptor on bladder cancer cell growth. Results Although leptin was not detected in the bladder tissue specimens, a decreased expression of the leptin receptor was observed in most cancer tissue specimens we analyzed. Furthermore, the forced expression of the leptin receptor in T24 bladder cancer cells prevented them from entering the S phase. Conclusions Our data demonstrated for the first time that the leptin receptor is aberrantly expressed in bladder cancer tissue and is possibly involved in the carcinogenesis of bladder cancer.

Vaccine (33)


http://www.sciencedirect.com/science/article/B6TD4-476W4JB-1R/2/acceccbed4d0a871d521cd36f27c2df5

The polymerase chain reaction method (PCR) has been applied to the diagnosis of foot-and-mouth disease viral RNA in tissues and, particularly, oesophageal-pharyngeal fluid (probang)
samples from cattle. Using primer sets which corresponded to conserved regions of the VP1 sequence of the viral genome, it was possible to amplify sequences regardless of the serotype/strain of the virus. In comparison with infectivity assays, the PCR was generally more sensitive although there were a number of examples where only infectivity was detectable. In experiments with uninfected probang samples deliberately seeded with a dilution series of virus, the PCR proved to be approximately 104 times more sensitive than infectivity assays. This greater sensitivity was attributed, in part, to the ability of the PCR to amplify specifically from non-infectious RNA preparations. This enabled the identification, by sequencing, of viral RNA from chemically inactivated virus concentrates typical of those used for commercial vaccine production. Amplification of specific PCR products was also achieved with virus eluted from commercial vaccine, including preparations which had been stored for more than 10 years at 4[deg]C. The PCR technique is of considerable value, therefore, both as a complement to infectivity assays and as a powerful tool in vaccine-associated studies.


http://www.sciencedirect.com/science/article/B6TD4-4BJDCVS-1/2/d775c7f62c37bf361274118bc63204ab

A post hoc analysis was performed using combined data from two Phase I tolerability/immunogenicity studies of monovalent human papillomavirus type 11 (HPV11) or HPV16 L1 virus-like particle (VLP) vaccines. The goal was to determine if the HPV16 L1 VLP vaccine protected against HPV16 infection. Vaccine or placebo was given at 0, 2 and 6 months. HPV16 infection was defined by positive polymerase chain reaction (PCR) results following vaccination. The incidence of HPV infection was observed to be 0 cases per 100 person-years at risk in the vaccine group, and 5 cases per 100 person-years at risk in the control group. These results support the institution of larger efficacy trials for HPV L1 VLP vaccines.


http://www.sciencedirect.com/science/article/B6TD4-47T1S71-2/2/3ce53320cfa04cee9d00fc1379225648

Outer membrane protein (OMP) vaccines are being developed against Neisseria meningitidis serogroup B which may provide protection against common circulating serotypes and serosubtypes in some countries. However, limited data is available in Europe from genosubtyping meningococci. We therefore undertook a retrospective analysis of the three main variable regions, VR1, VR2 as well as VR3, of the porA gene from N. meningitidis isolated from different countries, mainly from Scotland and Sweden. Analysis of this gene showed that, amongst 226 strains studied, there were a total of 78 different strains. No new VR1 or VR2 alleles were found but five new VR3 alleles are described. Our data indicates the importance of analysing the VR3 region of PorA in addition to VR1 and VR2 and also highlights, in general terms, the need for genosubtyping meningococci. Such analyses have major implications for the design of new meningococcal vaccines.


http://www.sciencedirect.com/science/article/B6TD4-48J4K43-1/2/1d3efbbaa79f9506bd48e8036898d32

The New World primate Aotus nancymaae is susceptible to infection by the human malaria parasite Plasmodium vivax and has therefore been recommended by the World Health Organization as a model for malaria vaccine candidate evaluation. We report the isolation, adaptation, titration and genetic characterization of a P. vivax wild strain in splenectomized A. nancymaae monkeys. Parasitemia remained high after 22 passages, reaching 7.88% by Giemsa and Acridine Orange staining and Real-Time PCR determination, making this P. vivax strain a highly infective and reliable asset to be used in P. vivax biological studies and vaccine development.


http://www.sciencedirect.com/science/article/B6TD4-3Y6Y3MY-J/2/feabdd71820a2989f2fc4762ed65c7c8

 Arenaviruses are emerging pathogens known to infect via the mucosa, however no formal attempts to make mucosal vaccines have been undertaken. Here we describe a recombinant aroA attenuated Salmonella typhimurium that expresses the nucleoprotein (NP) gene of Lassa fever virus (LAS). The complete NP gene was cloned downstream of the bacterial groEL promoter and integrated into the aroA locus of S. typhimurium. Lassa NP protein was detected in whole cell extracts from the recombinant Salmonella by immunoblot analysis with serum from Lassa-infected people. Mice were inoculated by intragastric intubation with 5 x 10^9 S. typhimurium and boosted with the same recombinant Salmonella 21 days after the primary inoculation. Both local mucosal IgA and serum immunoglobulins against Lassa NP were observed. Splenic cytotoxic T-lymphocyte responses to LAS NP were detected after the boost and they cross-reacted with target cells infected with the related arenavirus, lymphocytic choriomeningitis virus. Recombinant Salmonella elicits humoral and cell mediated immune responses against Lassa fever virus in mice and should be considered as a potential vaccine strategy in man.


http://www.sciencedirect.com/science/article/B6TD4-458P8WG-6/2/b1b975ed22ba05e30646101099a14ce4

The cellular components present in chlamydial preparations may contribute to the course of the experimental infection. NIH/S mice were inoculated and reinoculated intranasally with Chlamydia pneumoniae or a cellular preparation. The mock inoculation induced only mild histological changes in the lungs, which possibly induced partial protection against subsequent C. pneumoniae infection and, when given as reinoculation, possibly reactivated the culture-negative infection as culture-positive. In addition, serum antibodies against mouse heat shock protein 60 (Hsp60) were found in a few mice. In conclusion, the main immunopathogenic factors in a C. pneumoniae mouse model are chlamydial components. However, a cellular preparation may participate in an inflammatory reaction. Autoimmunity against Hsp60 may also play a role in the
pathogenesis of C. pneumoniae infection.


http://www.sciencedirect.com/science/article/B6TD4-40JFY80-7/2/684b0976558031de9fe725d609fdcc

Feline immunodeficiency virus (FIV) is a natural lentiviral pathogen of cats which can be experimentally transmitted via rectal and vaginal routes -- the major routes of human immunodeficiency virus type 1 transmission in man. An important objective for lentiviral research is the development of vaccine strategies which generate good mucosal immune responses capable of giving protection from a mucosal virus challenge. The experimental vaccines employed in this study were based on (a) a peptide from the third variable region of the FIV envelope glycoprotein and (b) fixed whole FIV, Glasgow-8 strain. Adjuvants used were Quil A and cholera toxin for mucosal administration and incomplete Freund's adjuvant and immune stimulating complexes for subcutaneous injection. Mucosal immunization was given by rectal and intranasal routes. Both antibody and proliferative responses were elicited by mucosal immunization and cholera toxin was found to be a good mucosal adjuvant. The addition of a lipothioester to the FIV peptide improved IgG and IgA responses upon parenteral administration. However, no protection from a rectal FIV challenge was achieved.


http://www.sciencedirect.com/science/article/B6TD4-3XRY69C-J/2/4235198135696d20615ccff99bb8692be

Cytotoxic T-lymphocyte (CTL) response is an important component of anti-viral immunity. CTLs are specific to short peptides presented by MHC-I molecules and immunisation with the exact peptide sequence introduced in the cytosol is therefore a minimal approach, which potentially affords a high degree of controllability. We have examined the induction of murine CTL's by this approach using DNA plasmid minigene vaccines encoding known mouse Kk minimal CTL epitopes (8 amino acids) from the influenza A virus hemagglutinin and nucleoprotein. We here report that such an approach is feasible and that wild type influenza virus flanking amino acid sequences can influence the CTL response but are not essential for optimal CTL induction. We also examined the effect of different new amino acid sequences flanking the CTL epitopes. In one version, two CTL epitopes were linked together as 'string of beads'. This did not improve CTL induction. In another version, one CTL epitope was inserted into a known T-helper protein (HBsAg). This did significantly augment the response probably due to immunological help from HBsAg Th epitopes. Finally, the CTL inducing minigene DNA vaccines were compared with Flu-induced CTL responses and tested for their protective effect against a lethal influenza A virus infection in mice and no effect was found. We conclude that a specific and highly directed CTL induction is possible by unlinked minigene DNA immunisation, but that CTL induction solely is not always sufficient to provide protection.

The parasitic nematode, Onchocerca volvulus is a major cause of blindness and dermal pathology in tropical regions. A vaccine directed to infective larvae would provide a valuable control tool alongside the current methods of chemotherapy and vector control. Previously we have described the identification of a chitinase cDNA that is expressed in a stage specific manner by O. volvulus infective third stage (L3) larvae. To evaluate its host protective potential, the complete open reading frame was cloned into the eukaryotic expression plasmid pJW4303 and used to vaccinate mice by DNA immunisation with the Accell GeneGun. The survival of challenge infective larvae was monitored using implanted micropore chambers. In the first trial, mice immunised 3 times over 4 months with 1 \( \mu g \) O. volvulus chitinase DNA responded with modest antibody responses dominated by IgG2a and exhibited a 36% (\( p=0.189, \) NS) reduction in parasite survival compared with challenge controls. In the second trial, an increased dose of DNA (5 \( \mu g \)) and more frequent immunisations (5 times over 6 months) stimulated an IgG1 dominant response and a 53% reduction in parasite survival (\( p=0.042 \)). Antibodies from the vaccinated mice reacted with the cuticle of post-infective L3 larvae, implying that this may be the site of immune attack following secretion of chitinase.


The effect of universal hepatitis B vaccination on the prevalence of serologically negative hepatitis B virus infection (HBV) and the emergence of HBsAg variants is unknown. We prospectively studied two different cohorts of 12-24 month old children in South Africa. They consisted of the unvaccinated children (n=459) born before the introduction of universal vaccination and the vaccinated children (n=1213) between 1 and 2 years after the introduction of the vaccination program. The frequency of detecting HBV DNA by PCR was reduced from 6.5% in unvaccinated children to 0.3% in vaccinated children (\( P<0.00001 \)). There were no unique amino acid substitutions within the major hydrophilic region of the S sequence in both pre- and post-vaccination samples. Universal childhood vaccination reduced the frequency of serologically negative HBV infection and did not necessarily lead to selection of escape variants.


Recombinant baculoviruses expressing the structural proteins of Venezuelan equine encephalitis virus (VEE) have been constructed and the intracellular processing, antigenicity, and immunogenicity of the expression products have been assessed. Baculoviruses expressing the entire structural protein region (C-E3-E2-6K-E1), or the complete glycoprotein region (E3-E2-6K-E1), generated products in Sf9 cells that were accurately and completely processed, and resulted
in mature proteins that were antigenically and electrophoretically indistinguishable from authentic viral proteins. These products were highly immunogenic in BALB/c mice, induced efficient VEE neutralizing responses, and protected these animals against challenge with virulent VEE. Expression of individual glycoprotein regions (E3-E2 and 6K-E1) generated products that were accurately but incompletely processed, and induced non-neutralizing antibodies that reacted more efficiently with denatured than native VEE proteins. Nonetheless, immunization with the 6K-E1 expression product provided complete protection against VEE challenge.


http://www.sciencedirect.com/science/article/B6TD4-464P50V-5/2/e0c9b5fca50359c1fc16f56ca19fc34

The antigenic variation of influenza A virus hemagglutinin (HA) and neuraminidase (NA) glycoproteins requires frequent changes in vaccine formulation. The classical method of creating influenza virus seed strains for vaccine production is to generate 6+2 reassortants that contain six genes from a high-yield virus, such as A/PR/8/34 (H1N1) and the HA and NA genes of the circulating strains. The techniques currently used are time-consuming because of the selection process required to isolate the reassortant virus. We generated the high-yield virus A/PR/8/34 (H1N1) entirely from eight plasmids. Its growth phenotype in embryonated chicken eggs was equivalent to that of the wild-type virus. By using this DNA-based cotransfection technique, we generated 6+2 reassortants that had the antigenic determinants of the influenza virus strains A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), A/teal/HK/W312 (H6N1), and A/quail/HK/G1/97 (H9N2). Our findings demonstrate that the eight-plasmid system allows the rapid and reproducible generation of reassortant influenza A viruses for use in the manufacture of vaccines.


http://www.sciencedirect.com/science/article/B6TD4-3Y44SNT-39/2/c31b1ad2107fbe8b3106a55a6aa01626

Shortly after infection of two rhesus monkeys (Macaca mulatta) either with a SIVmac32H challenge stock or with the same virus that had been passaged in another rhesus monkey for 11 months, SIV-envelope genes were cloned from their peripheral blood mononuclear cells and subsequently expressed by recombinant vaccinia viruses. The molecular weights and antigenicities of the thus produced envelope glycoproteins were largely identical to those of the native SIV. The envelope glycoprotein derived from the in vivo passaged virus proved to be poorly recognized by virus neutralizing monoclonal antibodies directed against one of the seven antigenic sites for which monoclonal antibodies were available. Immunization studies in rats showed that this protein was also less efficient in inducing antibodies against this antigenic site, and that it induced significantly lower levels of virus neutralizing antibodies than the other SIV-envelope glycoprotein. The immunogenicity of the SIV-envelope glycoprotein incorporated into immune stimulating complexes (iscoms) was compared to that of the same protein presented with Quil A or MDP-tsl.
Strain-specific nucleotide sequences of E1 and NS4 genes in five strains of a live rubella virus vaccine manufactured in Japan were identified for comparison, using 2389 nucleotides (1443 nucleotides of the E1 gene, 41 of the 3’ terminal region following the E1 gene and 905 of the NS4 gene). Sequences of the E1 gene in three strains (Matsuura, TCRB19 and To-336) were identified. Takahashi and Matsuba strains shared common sequences, but were discriminated by the sequence of the NS4 gene. These five strains showed a phylogenetic relationship with the places of their isolation. In a comparative study of three strains with their unattenuated progenitors, the nucleotides in these regions were almost conserved during the attenuation process.

Neutralising antibodies specific for feline leukaemia virus (FeLV) were induced by immunisation with recombinant FeLV transmembrane envelope protein p15E. Epitope mapping revealed two epitopes located in similar regions to those previously identified for the porcine endogenous retrovirus (PERV). One of the epitopes has partial homology and both are located in regions corresponding to epitopes recognised by neutralising antibodies in patients infected with HIV-1.

The simian immunodeficiency virus is a retrovirus closely related to the human immunodeficiency viruses; it induces an AIDS-like disease in macaques, and provides therefore an obvious animal model for anti-lentiviral drug and vaccine strategy assessments. In our experiment, we immunized rhesus macaques with a purified and formalin-inactivated whole SIVmac251 antigen preparation. Most of these monkeys were still protected for more than 4 months following a heterologous SIVsm intravenous challenge. Both virus stocks, for vaccine preparation and challenge, were provided by culture supernatants of infected T cells of human origin. Four of the protected macaques were then reimmunized with the same antigen preparation and rechallenged intravenously with a homologous rhesus cell grown SIVmac251. Unexpectedly, all animals developed clinical and biological evidence of infection by day 15 after the second challenge.
Inactivated influenza vaccine was administered intranasally to BALB/c mice together with an adjuvant (cholera toxin B subunit [CTB] supplemented with a trace amount of the whole toxin, CTB*) and its ability to induce innate immunity and confer protection against influenza was examined. Nasal wash virus titres 3 days after inoculation of homologous viruses were measured as an index of the ability of the vaccine to confer protection in mice immunized with either CTB*-combined vaccine or CTB* alone 1-21 days previously. The results were as follows. (1) Partial but significant reduction of the nasal-wash virus titres (prevention) was detected beginning 3 days after the vaccination, that is, 2 days earlier than the appearance of both virus-specific antibody-forming cells (AFCs) in the nasal-associated lymphoid tissue (NALT) and virus-specific IgA antibody responses in the nasal washes of mice immunized with the CTB*-combined vaccine. (2) The protection, detected on day 3 and peaking on day 5 but lost by day 21, was also conferred in mice immunized with CTB* alone. (3) The non-specific prevention was detected at doses of more than 0.3 [μg] of CTB*/mouse. (4) The nonspecific protection beginning 3 days after the immunization involved the enhanced expression of cytokine mRNAs (IL-15 and IL-18), considered responsible for natural killer (NK) cell activation, by the non-T cell populations in the NALT. (5) Normal NALT cells, when cultured in vitro with CTB*, secreted IL-1[beta] within a few hours in culture. These results demonstrate that the CTB*-combined vaccine, when given intranasally into mice, can confer nonspecific protection against influenza beginning 3 days after the vaccination and that CTB* also possessed this ability to confer protection non-specifically and temporarily by inducing the secretion of IL-1[beta], one of the most important cytokines that initiates both innate and adaptive immunity, and also NK cell activity.


http://www.sciencedirect.com/science/article/B6TD4-49RCXR6-D/2/31dcc85cdf15c178e49deb4255443d15

The widely administered Mycobacterium bovis BCG is an attractive live vector for the development of AIDS vaccines. We explored immune responses induced in cynomolgus macaques to rBCG-SIV3, a mixture of three recombinant BCG strains expressing the SIVmac251 nef, gag and env genes. After a single intradermal (ID) inoculation, circulating blood cells from rBCG-SIV3-vaccinated monkeys exhibited CTL responses targeted against the three antigens and interferon-gamma (IFN[gamma]) secretion was observed. A rectal or oral boosting dose of rBCG-SIV3 elicited anti-SIV IgAs in the rectum of vaccinated monkeys and increased IFN[gamma] secretion by circulating blood cells. Despite a good response against the vector, rBCG-SIV3 administration did not induce IgG antibody responses or lymphoproliferation against the SIV antigens in blood. This could be due to the lack of in vivo persistence of the recombinant BCG strains that were used. Rectal challenge with fully pathogenic SIVmac251-infected all animals. However, after viral challenge, anti-SIV cellular and antibody responses were higher in rBCG-SIV3 monkeys than in controls indicating that the vaccine induced anti-SIV CD4+ T-cell memory.


http://www.sciencedirect.com/science/article/B6TD4-45C0134-1/2/817d5f7c1e2ac4d7060147399f67188

We previously reported that the intracutaneous injection of DNA vaccines encoding Helicobacter
pylori heat shock proteins elicited specific immune responses, and led to reduced infection in mice. In this study, we constructed DNA vaccine encoding H. pylori-catalase (pcDNA3.1-kat) and investigated the immune responses to intranasal and intracutaneous administration of pcDNA3.1-kat. C57/BL6 mice were immunized intracutaneously with 10 [mu]g of pcDNA3.1-kat or intranasally with 50 [mu]g of pcDNA3.1-kat. Catalase-specific IgG antibody was detected in the sera of intranasal and intracutaneous immunized mice. Both intranasal and intracutaneous immunized mice were significantly protected from colonization by H. pylori and had significantly reduced degrees of gastritis. These results demonstrate that DNA vaccine encoding H. pylori-catalase can induce an immune response against H. pylori, and that intranasal immunization works as well as intracutaneous immunization.

http://www.sciencedirect.com/science/article/B6TD4-4FG8859-4/2/098675861b881c92914ffae51deb0f86

Dengue fever is a growing public health concern around the world and despite vaccine development efforts, there are currently no effective dengue vaccines. In the present study we report the induction of protective antibodies against dengue virus by DNA immunization with domain III (DIII) region of the envelope protein (E) in a mouse model. The DIII region of all four dengue virus serotypes were cloned separately into pcDNA 3 plasmid. Protein expression was tested in COS-7 cells. Each plasmid, or a tetravalent combination, were used to immunize BALB/c mice by intramuscular route. Presence of specific antibodies was evaluated by ELISA, and neutralizing antibodies were tested using a cytopathogenic effect (CPE) inhibition assay in BHK-21 cells, as well as in newborn mice challenged intracranially with dengue 2 virus. Mice immunized with individual DIII constructs or the tetravalent formulation developed antibodies against each corresponding dengue serotype. Antibody titers by ELISA were similar for all serotypes and no significant differences were observed when boosters were administered, although antibody responses were dose-dependent. CPE inhibition assays using Den-2 virus showed neutralization titers of 1:10 in mice immunized with individual DIII plasmid or those immunized with the tetravalent formulations. 43% of newborn mice challenged with Den-2 in combination with sera from mice immunized with Den-2 DIII plasmid were protected, whereas sera from mice immunized with the tetravalent formulation conferred 87% protection. Our results suggest that DIII can be used as a tetravalent DNA formulation to induce neutralizing and protective antibodies against dengue virus.

http://www.sciencedirect.com/science/article/B6TD4-3VGDCJX-8/2/d5876eb212c7b7503a4d2b2ae183fe46

Genes encoding the glycosylated precursor of the membrane (prM) and envelope (E) proteins of a Korean strain of Japanese encephalitis virus (JEV) were inserted into the genome of the host-range restricted, highly attenuated, and safety-tested MVA strain of vaccinia virus. MVA recombinants containing the JEV genes, under strong synthetic or modified H5 vaccinia virus promoters, were isolated. Synthesis of JEV prM and E proteins was detected by immunofluorescence microscopy, flow cytometry, and polyacrylamide gel electrophoresis. Mice inoculated and boosted by various routes with either of the MVA recombinants produced JEV neutralizing antibodies, that had titers comparable with those induced by an inactivated JEV vaccine, as well as haemagglutination-inhibiting antibodies. Mice immunized with 2 x 106
infectious units of MVA/JEV recombinants by intramuscular or intraperitoneal routes were completely protected against a 105 LD50 JEV challenge at 9 weeks of age.


http://www.sciencedirect.com/science/article/B6TD4-43G2YV1-V/2/7876d810b8bf2ce5849613e0317c6039

Vaccination with oncogene-derived DNA for anti-cancer treatment carries a risk of de-novo tumor induction triggered by the persisting recombinant DNA. We hypothesized that an oncoprotein whose primary sequence has been rearranged ('shuffled') to maintain all possible T cell epitopes still induces cytotoxic T cells against the authentic protein but is devoid of transforming properties. As a model antigen, we used the E7 oncoprotein of the human papillomavirus (HPV) type 16, the major cause of cervical cancer. We have generated an artificial E7 molecule in which four domains were rearranged and, in order to maintain all possible T cell epitopes, certain sequences were duplicated. Upon transfection of this shuffled E7 gene (E7SH) into RMA cells, presentation of an E7 Db-restricted T cell epitope was shown by an E7-specific CTL line in vitro. Immunization of C57BL/6 mice with E7SH DNA induced E7-specific CTL and also conveyed protection against E7-positive syngeneic tumor cells. No transforming activity of E7SH DNA in NIH3T3 cells was detected, as determined by focus formation, induction of S-phase under conditions of serum deprivation and degradation of endogenous pRB. Our results suggest that DNA shuffling may become a promising concept for DNA-based anti-cancer vaccines.


http://www.sciencedirect.com/science/article/B6TD4-454T75N-2/2/7f7ceb0fcc5bef4e749f81fd84552d

The adjuvant activity of Flt3 ligand (Flt3L) and conjugation to an interleukin (IL)-1[beta] bioactive fragment were compared, either alone or in combination, for their ability to induce T- and B-cell responses to the HGP-30 peptide sequence (amino acids 86-115 of human immunodeficiency virus (HIV) gag p17). The efficiency of HGP-30/IL-1[beta] conjugation, Flt3L administration or both as adjuvants was examined and all were found to augment similar levels of delayed type hypersensitivity (DTH) responses. In contrast, significant antigen (Ag)-specific types 1 and 2 T-cell ELISPOT responses were induced only by the combination of adjuvants. Further, in vitro sensitization with HGP-30 selectively increased Ag-specific, type 1 T-cell and cytotoxic T lymphocyte (CTL) responses to HGP-30-derived nonapeptide epitopes, while type 2 responses declined as measured in the ELISPOT assay. No serum antibodies to HGP-30 were induced unless HGP-30 was conjugated to keyhole-limpet hemocyanin. This suggests that a combination adjuvant strategy using Flt3L and conjugation to a biologically active IL-1[beta] fragment may be used to preferentially increase type 1 T-cell and CTL responses to HIV-1 gag antigenic epitopes.


http://www.sciencedirect.com/science/article/B6TD4-3Y44SNT-
We have reported previously the production of Plasmodium falciparum transmission-blocking monoclonal antibodies (mAb) recognizing a reduction-insensitive cross-reacting epitope in the gametocyte antigen Pfg27 and the gamete surface antigens Pfs230 and Pfs48/45. In this study, the amino acid sequence of this epitope in Pfg27 was determined. First, the epitope was localized near the N terminus of the protein by probing recombinant overlapping fragments spanning Pfg27 with transmission-blocking mAb in immunoblot experiments. The amino acid sequence of the epitope was then determined by using overlapping synthetic peptides spanning the smallest immunoreactive recombinant fragment in an ELISA. The sequence KPLDKFGNIYDYHYEH (amino acids 10-25 in the Pfg27 sequence) was shown to contain two overlapping epitopes recognized by transmission-blocking mAb. Comparison of the sequence of the gene encoding Pfg27 in seven different P. falciparum strains demonstrated that these sequential epitopes are totally conserved. Immunization of mice with synthetic peptides derived from Pfg27, conjugated with keyhole limpet hemocyanin (KLH) and formulated in Freund's adjuvant or alum, resulted in the production of antibodies capable of recognizing the peptides as well as the native Pfg27.


http://www.sciencedirect.com/science/article/B6TD4-4D1YV3C-4/2/bd95d461868f72d2212bc77dabb0457761

The product of the Bordetella bronchiseptica pertactin gene, prn, has been implicated as an adhesin and a protective immunogen in swine. Recent studies demonstrate prn sequence heterogeneity in swine isolates and vaccine strains within and surrounding the region 1 amino acid repeat GGXXPn and the region 2 amino acid repeat PQPn. However, only a few isolates have been evaluated. Allelic variation between vaccine strains and field isolates may affect vaccine efficacy, since region 2 is known to encode an immunodominant protective epitope. In the present study, the DNA and predicted amino acid sequences of the pertactin repeat regions from a collection of 81 recent swine field isolates and 5 vaccine strains from the United States were determined. Two region 1 variants and four region 2 variants, one of which has not been previously reported, were identified, comprising four pertactin types. Four vaccines are derived from strains with a region 1 variant identical to that found in the majority of field isolates. However, only two vaccines possess the most commonly identified sequence in region 2, while two others contain a variant found in only one other swine isolate. Ribotype analysis demonstrated that although vaccines containing the novel region 2 variant fall within the same major cluster as other common swine ribotypes, they are less closely related. No relationship was observed between pertactin type and ribotype.


http://www.sciencedirect.com/science/article/B6TD4-483BMS0-1/2/5ad6e9277a82f8856e01641d31a1b120

Regional recruitment of dendritic cells (DCs) by the local administration of granulocyte macrophage-colony stimulating factor (GM-CSF) or Flt3-ligand (Flt3L) has vaccine adjuvant activity. However, Flt3L, with its DC growth factor activity, has not been extensively studied as a vaccine adjuvant, particularly as a plasmid vector. We report that the intramuscular (IM) injection
of a Flt3L plasmid (pNGVL-hFlex), when formulated in a pluronic carrier (SP1017, Supratek Pharma, Inc., Laval, Que., Canada), recruits DC to the injection site and regional lymph nodes (LNs) and augments immune responses to a p17 HIV plasmid vaccine to a greater extent than the injection of a naked DNA vaccine alone. Following IM administration of pNGVL-hFlex, Flt3L mRNA, Flt3L protein and infiltrating DC accumulate at the injection site. The number of DC in the draining LNs are also significantly increased with the greatest increase observed following injection of 2.5 [mu]g of pNGVL-hFlex formulated in 0.01% SP1017. Flow cytometric studies demonstrate that the LN-infiltrating DC is mainly of the CD11c+CD11b- phenotype (IL-12 producing). Further, the co-injection of pNGVL3-hFlex and p17 HIV plasmids, formulated in SP1017, significantly increases the immune responses to the plasmid vaccine (pVAX-gag). The co-injection of pVAX-gag and pNGVL3-hFlex, formulated in SP1017, significantly increase delayed-type hypersensitivity responses and the numbers of antigen (Ag)-specific interferon-[gamma] secreting T cells in the spleen (Enzyme Linked Immune Spot (ELISpot) assay), compared to mice immunized with pVAX-gag formulated in SP1017 alone. We conclude that the IM injection of pNGVL-hFlex with SP1017 can increase the number of DC in draining LN and at the site of injection, thereby providing adjuvant activity for a plasmid vaccine resulting in a significantly increased, Ag-specific T cell response.


http://www.sciencedirect.com/science/article/B6TD4-3Y0J1DX-F/2/4aa4d35072b0113ef06d9488843ff76c

In a murine model of experimental cutaneous leishmaniasis, we investigated the protection elicited by injection of histone H1 isolated from parasites by perchloric extraction, of a H1 recombinant protein produced in E. coli, and of H1 long and short synthetic petides, against infection by L. major. Partial protection was achieved in most of the animals as shown by reduction in lesion size, upon immunization with histone H1 or its peptides, provided that the region 1-60 was present in the molecule. These observations argue in favor of a thorough examination of the possibility of including histone H1 described here in a cocktail vaccine against human leishmaniasis.


http://www.sciencedirect.com/science/article/B6TD4-48CFGNJ-2/2/4dacec4a86a5fda72da79aa5e66c30a9

Since virus-specific cytotoxic T lymphocytes (CTLs) play a critical role in preventing the spread of hepatitis C virus (HCV), an effective HCV vaccine should be capable of eliciting HCV-specific CTLs. In the present study, we assessed the capability of a novel recombinant vaccine using an attenuated tuberculosis bacillus, Calmette-Guerin bacillus (BCG), as a vaccine vehicle to elicit HCV-specific CTLs. BCG was engineered to express the CTL epitope of HCV-non-structure protein 5a (NS5a) as a chimeric protein with alpha antigen of mycobacteria. Immunization with this recombinant BCG elicited major histocompatibility complex class I-restricted CD8+ HCV-NS5a-specific CTLs in mice. Immunized mice showed a substantial reduction in the vaccinia virus titer compared with control mice when the immunized mice were challenged with a recombinant vaccinia virus expressing HCV-NS5a genes. These findings provide evidences for the possibility of BCG as a vaccine vector and its continued exploration as a vehicle for eliciting HCV-specific immunity.
Vanegas, R. A., N. E. Street, et al. (1997). "In a vaccine model, selected substitution of a highly stimulatory T cell epitope of hen's egg lysozyme into a Salmonella flagellin does not result in a homologous, specific, cellular immune response and may alter the way in which the total antigen is processed." Vaccine 15(3): 321.

http://www.sciencedirect.com/science/article/B6TD4-3RJX9N5-F/2/ab29550391320d85682e55cc5f1bb584

A 13 amino acid peptide corresponding to a potent BALB/c mouse T cell epitope of hen's egg lysozyme (HEL) was substituted singly at five sites in the d flagellin of Salmonella muenchen. The resulting chimeric proteins were unable to expand T cells capable of being stimulated by the HEL epitope and induced T cell populations which either failed to respond or responded at a low level to a normally highly stimulatory flagellin T cell epitope that was present in all chimeras. The results suggested that substitution of a T cell epitope in flagellin may alter the processing of the resulting immunogen.


http://www.sciencedirect.com/science/article/B6TD4-4F37HJM-1/2/f40668a399c5cb372be17349c72e3516

A DNA vaccine was tested in infant Rhesus macaques to evaluate its safety, immunogenicity and protective efficacy. Monkeys were vaccinated and challenged with a clinical isolate of human RSV. Vaccinated animals developed humoral and cellular responses following inoculation with plasmid DNA encoding the fusion (F) and nucleoprotein (N), from closely related bovine RSV. Vaccinated monkeys had decreased RSV in their lungs post-infection, and there was a qualitative difference in histopathology observed between vaccinated and unvaccinated animals. The combined result of safety and immunogenicity in a neonatal primate model is encouraging, suggesting the feasibility of DNA vaccines against RSV in infants.


http://www.sciencedirect.com/science/article/B6TD4-3VGDCRH-V/2/424ba987452b80cde96b1cbf92967d1a

Type 1 and type 2 immune responses are modulated by IL12 or IL4, respectively, at the time of lymphocyte priming. Importantly, type 1 responses have been associated with resistance to retroviral infection in mice, humans, and ruminants. Specifically, vaccination of sheep with vaccinia virus expressing bovine leukemia virus (BLV) gp51 resulted in protective immunity with the characteristics of a type 1 response, whereas vaccination of cattle resulted in a non-protective type 2 response. In order to test the hypothesis that cattle inoculated with BLV gp51 and IL12 will respond with a type 1 response, a recombinant vaccinia virus expressing BLV gp51 together with bovine IL12 was developed and characterized in vitro. For induction of type 2 responses a recombinant vaccinia virus expressing gp51 with bovine IL4 was similarly constructed and characterized. In this study recombinant cassettes were developed containing either the BLVenv gene alone or in combination with bovine IL4 or the two genes, p35 and p40, encoding bovine
IL12. Correct alignment with p7.5 or p11 vaccinia promoters and orientation was confirmed by complete sequencing. Recombinant vaccinia viruses were generated by homologous recombination, selected based on large plaque formation due to reconstitution of the vp37 gene, and structurally confirmed by Southern blotting. Transcription of recombinant BLVenv, bovine IL4, p35 and p40 was demonstrated by RT-PCR. Expression of BLVenv gp51 protein and bovine IL4 was shown by immunofluorescence and immunoblotting. Biologically active bovine IL4 expressed by vaccinia virus stimulated lymphoblast proliferation, B lymphocyte proliferation in the presence of CD40L, and inhibited IFN[gamma] secretion from PHA activated PBMC in a dose dependent fashion. Finally, bovine IL12 expression and biological function was confirmed by dose dependent induction of IFN[gamma] secretion by PHA activated PBMC and the moderate enhancement of lymphoblast proliferation. In conclusion, bovine IL12 and IL4 expressed by recombinant vaccinia virus in vitro clearly exhibited type 1-type 2 modulating properties.


http://www.sciencedirect.com/science/article/B6TD4-42NH3H1-N/2/2545758298e51d00769bfb242c467f84

The ability of inducing MHC class I restricted cytotoxic T lymphocytes response in vivo via recombinant filamentous phage was investigated. The recombinant filamentous phage particles that displayed the Hepatitis B virus epitope S28-39 were injected into BALB/c (H-2d) mice without adjuvants. A MHC class I restricted HBs specific CTL response was found 8 days after injection. The potentiality of using the recombinant filamentous phage as anti-virus vaccine was discussed.


http://www.sciencedirect.com/science/article/B6TD4-476MJYG-18/2/3f76d8345dcca71d6707489c69719110

We have used the potent mucosal immunogen cholera toxin (CT) to assess antigen-specific CD4+ T-cell responses, including Th1- and Th2-type cells in mucosa-associated tissues, e.g. Peyer's patches (PP), and systemic tissue, e.g. spleen (SP), for their regulatory role in the induction of CT-specific B-cell antibody responses in the gastrointestinal (GI) tract as well as in systemic sites. The CT was given by either oral or intravenous (i.v.) routes and the mice orally immunized with CT exhibited brisk IgA anti-CT antibody responses in faecal extracts and elevated IgG anti-CT antibody responses in serum. Further, significant IgA anti-CT spot-forming cells (SFCs) were seen in lamina propria lymphocytes (LPLs) from mice orally immunized with CT. In contrast, i.v. immunization with CT induced IgM and IgG anti-CT SFC responses in SP, and serum anti-CT antibodies of these two isotypes; no anti-CT responses were induced in the GI tract after immunization by this route. The CD4+ T cells isolated from PP and SP of mice orally immunized with CT were stimulated in vitro with CT-B-coated latex microspheres for 1-6 days, and the induction of IL-2 and interferon gamma (IFN-[gamma]) (Th1-type) or IL-4 and IL-5 (Th2-type) producing SFCs were analysed by a cytokine-specific ELISPOT and cytokine-specific mRNA was detected by reverse transcriptase (RT)-PCR assays. Cultures of PP and SP CD4+ cells from orally immunized mice contained numbers of IL-4- and IL-5-SFCs, significant levels of IL-4 and IL-5 mRNA, only small numbers of IFN-[gamma]- and IL-2-SFCs and low to undetectable levels of IFN-[gamma] and IL-2 mRNA. On the other hand, SP CD4+ Th-cell cultures from mice immunized i.v. with CT included both IL-4- and IL-5- as well as IFN-[gamma]- and IL-2-SFCs; no CT-specific Th cells were detectable in PP. These results show that oral immunization with CT preferentially induces CD4+ T cells which produce IL-4 and IL-5 in both PP
and SP. Furthermore, the presence of elevated numbers of these Th2-type cells directly correlated with significant IgA anti-CT-B antibody responses in LPLs isolated from the GI tract.

**Vascular Pharmacology** (1)


http://www.sciencedirect.com/science/article/B6X3P-4CBV1FG-1/2/78ca2e192905b534e537dca42d09bc57

We used a selective EP4 receptor agonist, ONO-4819, and a human leukemic T cell line MOLT-3 cells, which express all four prostaglandin E2 (PGE2) receptors (EP1-EP4), to investigate whether the EP4 PGE2 receptor subtype is involved in regulating lymphocytic cholinergic activity. Phytohemagglutinin (PHA), a T cell activator, significantly enhanced the expression of EP4 receptor mRNA during the first 3-6 h of exposure, after which, expression gradually declined. Furthermore, PHA stimulation slightly but significantly up-regulated the expression of EP2 mRNA after 12 h and of EP3 mRNA after 6 h. By contrast, expression level of EP1 receptor mRNA was not affected by PHA. ONO-4819 (1 [mu]M), which was added to cultures after 3 h of PHA stimulation, significantly increased cellular ACh content and release, and up-regulated ChAT mRNA expression and activity but inhibited MOLT-3 cell proliferation. These findings suggest that the activation of T lymphocytes up-regulates EP4 receptor mRNA expression and, to a lesser extent, EP2 and EP3 receptors and that PGE2 enhances nonneuronal lymphocytic cholinergic transmission in activated T cells, at least in part, via EP4 receptor-mediated pathways.

**Vet Rec.** (1)


http://veterinaryrecord.bvapublications.com/cgi/content/abstract/155/19/593

Cervical swabs and serum samples were taken from Swiss herds of sows with high rates of irregular return to oestrus (group A) and from control herds without reproductive problems (group B. The genital tracts of 21 slaughtered sows of group A were also examined. The swabs and genital tracts were screened for Chlamydiae by a new 16S rRNA PCR and the sera by an ELISA for Chlamydiaceae lipopolysaccharide. Chlamydophila (Cp) abortus was isolated from seven of the 65 swabs taken from group A but from none of the 128 swabs taken from group B. Chlamydia suis was present in swabs from both groups A (1.5 per cent) and B (2.3 per cent). In addition, Cp abortus was detected in 33.3 per cent of the genital tracts. Of the 193 sera tested, 61.7 per cent were positive, with no significant difference between group A (52.3 per cent) and group B (66.4 per cent). Chlamydia-like organisms were detected in 28.2 per cent of the swabs from group A
and in 22 per cent of those from group B.

**Vet. Pathol.** (4)


http://www.vetpathology.org/cgi/content/abstract/41/5/506

Bilateral chronic granulomatous nephritis and meningoencephalitis were diagnosed on necropsy of a 2-year-old male Great Dane dog. The causative agent was identified as Balamuthia mandrillaris, based on morphologic features, immunohistochemical staining, and deoxyribonucleic acid detection using the polymerase chain reaction with newly designed primer pairs. Trophozoite and cystic forms of the amoeba were evident within the kidneys and brain parenchyma. This is the first report on a B. mandrillaris infection in a dog.


http://www.vetpathology.org/cgi/content/abstract/42/1/42

Tissues and fecal material were collected from 14 North American bison (Bison bison) that were suspected of having Johne's disease and analyzed for the presence of Mycobacterium avium subsp. paratuberculosis (M. paratuberculosis). Sections of ileum, ileal-cecal lymph node, and three sequential sections of jejunum with their associated mesenteric lymph nodes were taken from each animal. Fecal culture indicated that 5 of 14 (35.7%) animals were infected, whereas cultures from tissues detected 12 of 14 (85.7%) animals as infected and 59 of 111 (53.2%) of the tissues as positive for M. paratuberculosis. Polymerase chain reaction analysis identified infection in 14 of 14 (100%) animals and in 91 of 112 (81.2%) tissues. In addition, tissues were processed for Ziehl-Neelsen acid-fast staining, auramine O/acridine orange fluorescent staining, and immunohistochemical staining. Ziehl-Neelsen and auramine O staining identified 7 of 14 (50%) and 5 of 14 (35.7%) animals as infected and 24 of 112 (21.4%) and 28 of 112 (25%) tissues as positive, respectively. Immunohistochemical analyses of bison tissues, using antisera collected from rabbits immunized with four different preparations of M. paratuberculosis, identified a greater percentage of infected animals (ranging from 57 to 93%) and positive tissues (ranging from 28 to 46%). Collectively, these data indicate that DNA-based detection of M. paratuberculosis was more sensitive than bacterial culture or staining, identified infection in all the bison, and detected the greatest number of positive tissues within each animal.


http://www.vetpathology.org/cgi/content/abstract/41/3/221
Heritable, type-2 von Willebrand's disease (vWD) was studied in a line of German Shorthaired Pointers (GSPs) in which some members had a nucleotide variant in exon 28 of the von Willebrand factor (VWF) gene. A polymerase chain reaction (PCR) diagnostic test for the nucleotide variant was developed to establish the disorder's mode of inheritance and to eliminate it from the line. Thirty-six of the 49 GSPs in the line, 14 unrelated GSP controls, and 71 unrelated dogs of various breeds were tested for the presence of the variant nucleotide. All the dogs with a VWF antigen deficiency (<70% of normal) were either homozygous or heterozygous for the nucleotide variant. The variant was not located in any tested dog in the line or outside of the line with a VWF antigen value greater than 68%. Of the GSPs in the line tested, two were homozygous for the variant, 15 were heterozygous, and 19 were variant free. The collective evidence of this and other studies is consistent with the variant nucleotide being the cause of the type-2 vWD in this line of GSPs and German Wirehaired Pointers. The PCR diagnostic test for the variant nucleotide was successfully used to select and produce progeny that were variant free and vWD free. This test should be effective in the subsequent elimination of this same variant from other lines of dogs.


http://www.vetpathology.org/cgi/content/abstract/41/4/326

We evaluated gene expression and antimicrobial responses of bovine monocyte-derived macrophages incubated with Mycobacterium avium subsp. paratuberculosis (M. a. ptb), the causative agent of Johne's disease. Gene expression was evaluated by the use of human noncompetitive high-density oligonucleotide microarrays. Bovine messenger RNA hybridized with 14.2-18.2% of the 12,600 oligonucleotide probe sets. When macrophages incubated with M. a. ptb were compared with nonactivated control macrophages, macrophages activated by addition of interferon-γ and lipopolysaccharide, and macrophages incubated with Mycobacterium avium subspecies avium (M. a. a), 47, 79, and 27 genes, respectively, were differentially expressed. Differential expression of six of these genes was confirmed using reverse transcriptase polymerase chain reaction. Several functional assays were performed to evaluate the potential relevance of differentially expressed genes to host defense. Macrophages phagocytizing M. a. a had a greater capacity to kill the organisms and to acidify phagosomes and a greater degree of apoptosis than did macrophages incubated with M. a. ptb. The results of these studies indicate that multiple genes and metabolic pathways are differentially expressed by macrophages ingesting mycobacterial organisms. Although the intracellular fate of mycobacterial organisms appears to be dependent on a complex interaction between macrophage and organism, phagosome acidification and apoptosis may play central roles in organism survival.

Veterinary Parasitology (22)

With the aim of developing more simple diagnostic alternatives, a differential single-round and multiplex polymerase chain reaction (PCR) method was designed for the simultaneous detection of Babesia caballi and Babesia equi, by targeting 18S ribosomal RNA genes. The multiplex PCR amplified DNA fragments of 540 and 392 bp from B. caballi and B. equi, respectively, in one reaction. The PCR method evaluated on 39 blood samples collected from domestic horses in Mongolia yielded similar results to those obtained from confirmative PCR methods that had been established earlier. Thus, the single-round and multiplex PCR method offers a simple tool for the differential diagnosis of B. caballi and B. equi infections in routine diagnostic laboratory settings as well as in epidemiological studies.


http://www.sciencedirect.com/science/article/B6TD7-3VYHT9-13/2/b9c82c78e5b5fd06b54361ad5e028b69

A nested polymerase chain reaction for detecting Ehrlichia equi in horses and ticks (Ixodes pacificus) was developed. A major second-round PCR product of 928 bp could be readily visualized in ethidium bromide-stained agarose minigels. An internal probe was used to verify the identity of the amplified product by non-radioactive (digoxigen-based) Southern blotting: additional confirmation was provided by DNA sequence analysis. A dilution study testing the sensitivity of the PCR indicated that DNA derived from 3 infected neutrophils was sufficient to generate a PCR signal. The specificity of the PCR was examined using a panel of rickettsiae, of which only E. equi and the closely-related human granulocytotropic ehrlichia produced PCR bands. In an in vivo infection study, E. equi DNA was detected in blood buffy-coat cells of an experimentally-infected horse on days three through 14 post-inoculation. In a separate study, three of six adult I. pacificus that as nymphs had been fed on an experimentally infected horse were found to be PCR-positive for E. equi.


http://www.sciencedirect.com/science/article/B6TD7-3RGSY66-9/2/288701958c8b315399b62700ef18bd3b

A nested polymerase chain reaction was developed for amplifying a 529-bp segment of the 16S ribosomal RNA gene of Ehrlichia risticii from equine buffy coat cells. Confirmation of identity of the amplified bands was accomplished by Southern hybridization and DNA sequencing. The study indicated a detection limit of > 10 copies of the target gene, and specificity for E. risticii as based on a panel of test rickettsiae. Ticks (Ixodes pacificus) collected in an area of northern California enzootic for equine monocytic ehrlichiosis were found to be negative for E. risticii DNA.


http://www.sciencedirect.com/science/article/B6TD7-44SHF4R-7/2/122727509d9e86cd16cd8f5297f313d1
Molecular evidence that suggests the possible role of the ixodid tick, Haemaphysalis longicornis and its eggs in the transmission of equine Babesia caballi parasites is presented herein. Using polymerase chain reaction (PCR) to assay for DNA in parasites, presumably acquired by ticks that were allowed to feed on splenectomized-SCID mice, experimentally exposed to in vitro-cultivated B. caballi, we have obtained positive bands that corresponded to the expected B. caballi-specific 430 bp gene fragment in 50% of female ticks used, and in 75 and 25% of eggs and larval progeny, respectively. Also, parasite DNA was detected in ticks, eggs and larvae as late as the 16th to the 20th day post-host infestation. Present findings support to the potential role of H. longicornis in the transmission of B. caballi parasites. Its capability, however, to successfully transmit the infection to horses under natural conditions in the field needs to be further ascertained. To our knowledge, this is the first documented study incriminating H. longicornis as a most and likely biological vector of equine babesias.


http://www.sciencedirect.com/science/article/B6TD7-49W1WSF-3/2/623875c7344c2d21fccc8131b1cd5965f

We present observations on an unusual tetratrichomonad species isolated from preputial smegma of virgin bulls. Ultrastructural studies were performed using scanning and electron microscopy techniques. This protozoan presents four anterior flagella of unequal length and a recurrent one forming the undulating membrane. It shows one anterior nucleus, a Golgi complex, an axostyle, and a costa. The hydrogenosomes are rather elongated, seen in groups, and presenting different electron densities. Vacuoles of different sizes containing bacteria and material in process of digestion were frequently found. PCR was also used in order to compare the species herein described with other trichomonad species. The amplification products were seen only with primers TFR1 and TFR2 (specific to trichomonads), but not with TFR3 and TFR4 (specific to Tritrichomonas foetus), suggesting that although collected from the genital tract of the bull, this protist was not T. foetus. We propose that the appearance of these tetratrichomonads were probably due to the sodomy practiced among bulls. Concomitant contamination of preputial cavity with feces could explain the presence of the opportunistic organism. The observations presented here show the importance of the correct diagnostic when investigating samples obtained from the urogenital tract of cattle. We also suggest that this flagellate belongs to the species Tetratrichomonas buttreyi.


http://www.sciencedirect.com/science/article/B6TD7-4177K34-1/2/c6ca5d9c0f344c054d6dcac60bd443de

An epizootiological survey of leishmaniosis, coccidiosis and parasitic helminths in 67 foxes (Vulpes vulpes) was conducted in Guadalajara (central Spain). Examination for parasitic protozoa revealed prevalences of 74% Leishmania (determined by molecular methods) and 2.9% coccidia oocysts (fecal flotation). Survey of parasitic helminths (fecal flotation/necropsy) demonstrated the presence of nine species, including six nematodes, two cestodes and one trematode. Nematodes were the most common parasites of foxes, followed by cestodes and trematodes. Greater levels of nematodes like Uncinaria, with a free-living stage in its life-cycle, were found in foxes in areas where moist soils were likely to exist, in contrast to areas of semiarid characteristics, where Toxascaris leonina or Trichuris vulpis were predominant. With regard to helminths of importance as human pathogens, trichinoscopy revealed the presence of a relatively high number of foxes
(8.9%) infected with Trichinella spiralis. Finally, Toxocara canis infection was less frequent (4.4%) than trichinellosis.


http://www.sciencedirect.com/science/article/B6TD7-48407K0-2/2/231d335adecab1886eab357f361bf03

Molecular epizootiology of piroplasmids (Babesia spp., Theileria spp.) and Hepatozoon canis was studied in mammals from southern Europe (mainly from Spain, but also from Portugal and France). Partial amplification and sequencing of the 18s rRNA gene was used for molecular diagnosis. In some particular cases (B. ovis and B. bovis) the complete 18s rRNA gene was sequenced. Blood samples were taken from domestic animals showing clinical symptoms: 10 dogs, 10 horses, 10 cows, 9 sheep and 1 goat. In addition, DNA samples were isolated from blood of 12 healthy dogs and from spleen of 10 wild red foxes (Vulpes vulpes). The results of the survey were the following:- Piroplasmid infections: Approximately from 50 to 70% of wild or domestic mammals (symptomatic) were infected.- Piroplasmids detected in ruminants were: Cow: B. bovis, T. annulata and Theileria sp. (type C). Sheep and goat: B. ovis.- Piroplasmids present in canids were: Babesia canis vogeli, Babesia canis canis, Theileria annae and B. equi. The only piroplasmid found in asymptomatic dogs was B. equi.- Piroplasmids found in horse were: B. equi and B. canis canis.- H. canis infections in canids: H. canis was absent of domestic dog samples, whereas all foxes studied were infected by this protozoa. Genetic analysis showed that most of piroplasmid and Hepatozoon isolates from southern Europe matched unambiguously with previously described species, as demonstrated by the high level sequence identity between them, usually between 99 and 100%. Minor differences, usually detected in hypervariable regions of 18s rRNA gene are probably due to strain variations or rare genetic polymorphisms. A possible exception was B. bovis, which shows a relatively lower degree of homology (94%) with regard to other B. bovis isolates from several countries. The same is true for B. ovis, that showed a 94% identity with regard to Babesia sp. from South African cow and a 92% with rapport to B. bovis from Portugal.


http://www.sciencedirect.com/science/article/B6TD7-3RJG29C-N/2/e230cbf8bca7ef06fd0d89a868d0db1c

Transstadial transmission of human granulocytotropic Ehrlichia (HGE) was attempted in dogs using Amblyomma americanum (L.) and A. maculatum Koch, two species that, as adults, feed readily on human beings. Larvae and nymphs were acquisition-fed on a dog that was parasitemic with HGE. Two months later, following digestion of the blood meal and subsequent molting to nymphal or adult stage, these ticks were fed to repletion on HGE-naive dogs. None of the dogs developed clinical evidence of ehrlichiosis. Parasites were not observed in blood smears by light microscopy, HGE DNA was not detected by polymerase chain reaction, and none of the dogs seroconverted. Based on this trial, we conclude that, unlike E. chaffeensis, HGE is probably not transmitted from dog to dog by either A. americanum or A. maculatum.
Pyrethroid resistance in three horn fly populations in Louisiana was monitored by weekly fly counts, filter paper bioassays, and diagnostic PCR assays for the presence of pyrethroid resistance-associated mutations in the sodium channel gene coding region. The PCR assay for the knockdown resistance (kdr) and superkdr sodium channel mutations was used to determine the frequency of the target site insensitivity mechanism in the populations of horn flies, which possessed varying degrees of insecticide resistance. The bioassays and frequency of homozygous-resistant (RR) kdr genotypes were relative predictors of the fly control subsequently observed. Flies exposed to filter paper impregnated with a discriminating concentration of one of four different insecticides were collected when 50% mortality was estimated. Genotypes for the dead flies and the survivors were determined by the PCR assay. The results of the PCR assays indicated that the genotype at the kdr locus of the flies exposed to the two pyrethroids had an effect upon whether the flies were considered to be alive or dead at the time of collection. The kdr genotype of flies exposed to either diazinon or doramectin was unrelated to whether the flies were considered to be alive or dead, except for a single comparison of flies exposed to diazinon. When possible interactions of the kdr and superkdr mutations were compared, we found that there were no associations with the response to diazinon and doramectin. For one location, there were no survivors of the 75 flies with the SS-SS (superkdr-kdr) homozygous susceptible wild type genotype exposed to pyrethroids, while there were survivors in all of the other five genotypes. The SS-RR genotype flies were more susceptible to the pyrethroids than the SR-RR flies, but that was not the case for exposure to diazinon or doramectin. In the St. Joseph population, there were an adequate number of flies to demonstrate that the SS-SR genotype was more susceptible to pyrethroids than the SS-RR and that flies with the SR-SR genotype were more susceptible to pyrethroids than the flies with the SR-RR genotype.


Tritrichomonas foetus (T. foetus) is the causative agent of bovine trichomonosis, a sexually transmitted disease leading to abortion (from 1 to 8 months gestation), infertility, and occasional pyometra. The annual losses to the U.S. beef industry are estimated to be in the hundreds of millions of dollars. Currently, the "gold standard" diagnostic test for trichomonosis in most countries is the cultivation of live organisms from reproductive secretions. The cultured organisms can then be followed by PCR assays with primers that amplify T. foetus to the exclusion of all other trichomonad species. Thus, negative results present as null data, indistinguishable from failed PCR amplification during T. foetus specific amplification. Our newly developed assay improves previously developed PCR based techniques by using diagnostic size variants from within the internal transcribed spacer 1 (ITS1) region that is between the 18S rRNA and 5.8S rRNA subunits. This new PCR assay amplifies trichomonad DNA from a variety of genera and positively identifies the causative agent in the bovine trichomonad infection. This approach eliminates false negatives found in some current assays as well as identifying the causative agent of trichomonad infection. Additionally, our assay incorporates a fluorescently labeled primer enabling high sensitivity and rapid assessment of the specific trichomonad species. Moreover, electrophoretic separation of amplified samples can be outsourced, thus eliminating the need for diagnostic laboratories to purchase expensive analysis equipment.

http://www.sciencedirect.com/science/article/B6TD7-44CHMK7-2/2/13254e59381fe7adad5290cdd7203a

A single tube nested polymerase chain reaction (PCR) assay targeting the multicopy 18S-5.8S rRNA internal transcribed spacer (ITS1) region has been developed for the diagnosis of Toxoplasma gondii-induced abortion in ovine fetal tissues. In all, 145 ovine fetal samples including brain, spleen, lung, liver, kidney, placenta and fetal fluids from 53 fetuses and stillborns of 32 farms in Northern Spain were analyzed. Thirty-six samples belonging to nine fetuses and one stillborn lamb were T. gondii PCR-positive. Although T. gondii DNA was amplified from different types of tissues, brain was the tissue with the highest detection rate. All animals that had histopathological lesions associated to T. gondii infection were positive by PCR. In addition, four fetuses whose histological examination was hindered by autolysis were PCR-positive. Results obtained by PCR and indirect fluorescent antibody test (IFAT) showed good correspondence, demonstrating the diagnostic value of the two techniques. However, PCR has the advantage over serology in its ability to diagnose T. gondii infection at earlier stages of gestation when the fetus is not yet immunocompetent and in lambs that have taken colostrum. Once other abortifacient agents are ruled out, PCR detection of the ITS1 region in fetal tissues is a valuable and relatively rapid technique for the diagnosis of ovine abortion caused by T. gondii.


http://www.sciencedirect.com/science/article/B6TD7-4F9FR21-1/2/8892f52245a2fa31e751aa5b5c3f5ee

The high degree of immunity induced by the bovine lungworm, Dictyocaulus viviparous, makes it an ideal model in which to study nematode-induced protective immune responses. Here, cytokine responses were measured over the course of an experimental infection of D. viviparous. Local cytokine messenger RNA (mRNA) transcripts were measured in lung parenchyma, tracheal rings and draining lymph nodes using semi-quantitative reverse transcriptase-polymerase chain reaction. Responses were measured in animals necropsied at 15, 22 and 43 days post-infection (DPI). The responses elicited at these time points were compared with cytokine levels observed in uninfected animals. Interleukin (IL)-4, IL-5, IL-10, IL-12p35, IL-13 and interferon gamma (IFN[gamma]) mRNA levels were measured in duplicate at each site. By 42 DPI, very few parasites were recovered, either from faeces or lungs. Transcripts of all cytokines increased in the lung parenchyma, tracheal rings and caudal mesenteric lymph nodes by 15 DPI. The response was rapid and peaked during the time of larval migration through the lungs. By 42 DPI, expression levels of most cytokines were reduced to levels similar to, or below, base line values measured in uninfected animals. Highest levels of IL-10, IL-12p35, IL-13 and IFN[gamma] transcript were measured in the bronchial lymph nodes of uninfected animals. IgG1 levels were negatively correlated with expression levels of all cytokines. The results demonstrate that a mixed cytokine response occurs over the course of a primary infection during which the parasites were eliminated by day 43 DPI. These results agree with those obtained for other helminths in cattle and challenge the hypothesis that polarised Th2 responses are essential for protection against nematodes in this species. These observations are important in the development of recombinant vaccines, particularly when considering adjuvant choice.

http://www.sciencedirect.com/science/article/B6TD7-458P8Y8-1/2/768fdd7fcdc10fe3c142671360e33b98

The astigmatid mite Psoroptes ovis is the causative agent of sheep scab, a highly contagious parasitic disease of sheep. Infection causes severe allergic dermatitis, resulting in damage to the fleece and hide, loss of condition and occasional mortality. Interest in the P. ovis allergens led us to characterise a glutathione S-transferase (GST) which displays homology to GST allergens isolated from the house dust mite, Dermatophagoides pteronyssinus and the cockroach, Blatella germanica. A cDNA encoding a mu-class GST from P. ovis was expressed in Escherichia coli and the recombinant protein purified for biochemical analysis. SDS-PAGE analysis indicated that the purified product was homogeneous and had an apparent molecular weight of 30 kDa. The recombinant GST (rGST) is active towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB), whereas 1,2-dichloro-4-nitrobenzene (DCNB) is a poor substrate. The recombinant protein was also tested for recognition by IgE and IgG antibodies in serum from P. ovis naive and P. ovis infested sheep. Neither IgE nor IgG antibodies were detected to the rGST. Prausnitz-Kustner testing with rGST did not provoke a characteristic weal and flare response. Biopsies collected at the PK test sites were stained for eosinophils, neutrophils, mast cells and basophils. Neutrophil, mast cell and basophil counts were not significantly different to the controls. Eosinophil numbers were significantly higher than controls, but were not due to an IgE response.


http://www.sciencedirect.com/science/article/B6TD7-3WV9GC7-7/2/d9817b446c71ef9325bf884814adb340

The need for alternative control strategies for sheep scab is critical. One approach is to develop vaccines based on 'concealed' antigens derived from Psoroptes ovis. This strategy requires the identification and characterisation of potential target antigens, which has been hampered by the problem of limited biological material for isolation of protein antigens. To aid the discovery of P. ovis antigens and to provide a resource for generating recombinant protein, we constructed a P. ovis cDNA expression library, using total RNA isolated from 250 mg of mixed-stage P. ovis and the Clontech SMART(TM) cDNA synthesis kit. The presence of P. ovis-specific sequences was confirmed using PCR amplification and sequencing of actin. The sequences of cDNA inserts from six random clones included one with high homology to the Dermatophagoides pteronyssinus (house dust mite) antigen p Dp15. This is a glutathione S-transferase known to be an important house dust mite antigen. We conclude that this library will be a useful tool for the identification of potential target antigens for the immunological control of P. ovis and to further our understanding of the pathology of sheep scab.


http://www.sciencedirect.com/science/article/B6TD7-4DDR7TJ-1/2/6f60dca5406d6d17757f82d22f8df39f

In this study, different types of tissue sampling for PCR-based diagnosis and follow-up of canine visceral leishmaniosis were compared. Skin, whole blood and lymph node samples were
collected from 95 naturally infected dogs living in South Italy, where the disease is endemic. Twenty-nine of these 95 dogs, treated with meglumine administered concurrently with allopurinol for 30 days, and then with allopurinol alone, were monitored during a period of 2 years. The DNA extracted from the clinical specimens was amplified by PCR using as target DNA a 116-bp fragment in the constant region of the kinetoplast DNA minicircle. PCR analysis was more sensitive than indirect immunofluorescence antibody test in detecting Leishmania infection in symptomatic dogs: 99% of lymph node samples resulted positive, whereas 94% of blood samples and 95% of skin samples gave a positive result. PCR analysis of samples from dogs followed up 2 years showed that: (1) all subjects resulted positive in at least one of the three types of samples; (2) all time the dogs had a relapse, PCR resulted positive in all three types of samples; (3) when dogs were apparently healthy, PCR analysis was positive on skin and lymph node samples, but not always on blood samples. Since lymph node sampling is invasive and sometimes difficult in healthy asymptomatic dogs, our results suggest that, independently from the presence or not of cutaneous lesions, skin biopsy represents a good substratum for PCR-based diagnosis and follow-up of canine visceral leishmaniosis.


http://www.sciencedirect.com/science/article/B6TD7-48HXRKR-3/2/93dc5b374016a6c0de7a41e73033c2e5

Diagnosis of the cutaneous form of canine leishmaniosis is mostly performed by histological or immunohistological examination of skin biopsies. In modern histology, the polymerase chain reaction (PCR) has gained increasing importance as a complementary tool to directly demonstrate the presence of parasite DNA in the tissue sections. For the present study, a previously described Leishmania-PCR has been further developed and optimised in view of its practicability for routine histological application. Since formalin-fixation of histological specimens causes partial DNA-destruction, which may hamper diagnostic PCR analysis, primers specific for the highly conserved [alpha]-actin gene sequences were used to pre-diagnostically assess the isolated sample-DNA for its functionality in a PCR-reaction. This [alpha]-actin-specific PCR detects DNA from a large variety of mammalian species and thus exhibits relevance for both human and veterinary medical application. A recombinant internal positive control was introduced to monitor possible sample-related inhibitory effects during the amplification reaction. We performed a retrospective evaluative study with 18 formalin-fixed samples from dogs with suspected or proven leishmaniosis. Six samples were PCR-incompatible. In turn, 9 of the other 12 samples were PCR-positive, and immunohistochemical results matched these findings. Based on these technical achievements, the Leishmania-PCR proved to be a valuable tool to complement conventional histological and immunohistological methods for diagnosis of cutaneous leishmaniosis in formalin-fixed, paraffin-embedded skin biopsies.


http://www.sciencedirect.com/science/article/B6TD7-3V51CF7-6/2/fba7015f04108e60c55c1eb7b14f443c

Polymerase chain reaction and Southern hybridization were used to survey for the presence of Ehrlichia canis, Ehrlichia chaffeensis, and Ehrlichia ewingii in blood samples of 65 dogs that harbored ticks from northcentral and northeastern Oklahoma. Dog blood samples were also examined for antibodies against E. canis and E. chaffeensis, using an immunofluorescent
antibody test. Ten of 65 dogs (15.4%) examined were positive for Ehrlichia spp. by PCR. Four (6.2%) were positive for E. ewingii, 2 (3.1%) for E. canis, and 4 (6.2%) for E. chaffeensis. Seven dogs (10.8%) were seropositive for E. canis or E. chaffeensis. Ticks collected from PCR-positive dogs were examined by PCR for the presence of Ehrlichia DNA. Several groups of ticks were PCR-positive for E. ewingii or E. canis. E. canis was detected in Rhiziphephalus sanguineus, which is considered the major vector for that organism. E. ewingii was detected in a larger variety of ticks, including the only known vector Amblyomma americanum, as well as in Dermacentor variabilis and R. sanguineus. Results suggest that Ehrlichia spp. which are canine and human pathogens circulate in dogs in Oklahoma and in several tick species that feed on dogs.


The coccidian parasite Neospora caninum is an intracellular protozoan, causing abortion in cattle in many countries around the world. In this study, the protective potential of the major N. caninum surface antigen NcSRS2, expressed in Escherichia coli and formulated into immunostimulating complexes (iscoms), was investigated in an experimental mouse model. The recombinant protein was specially designed for binding to iscoms via biotin-streptavidin interaction. Two groups of 10 BALB/c mice were immunised twice, on days 0 and 28 with iscoms containing either the recombinant NcSRS2 (NcSRS2 iscoms) or similar iscoms with NcSRS2 substituted by an unrelated recombinant malaria peptide (M5) as a control (M5 iscoms). A third group of 10 age-matched BALB/c mice served as an uninfected control group. Immunisation with recombinant NcSRS2 iscoms resulted in production of substantial antibody titres against N. caninum antigen, while the mice immunised with M5 iscoms produced only very low levels of antibodies reacting with N. caninum antigen. After challenge infection with N. caninum tachyzoites on day 69, mice immunised with NcSRS2 iscoms showed only mild and transient symptoms, whereas the group immunised with M5 iscoms showed clinical symptoms until the end of the experiment at 31 days post inoculation. A competitive PCR assay detecting Nc5-repeats was applied to evaluate the level of parasite DNA in the brain. The amount of Nc5-repeats in the group vaccinated with NcSRS2 iscoms was significantly lower than in the control group given M5 iscoms. In conclusion, it was found that the recombinant NcSRS2 iscoms induced specific antibodies to native NcSRS2 and immunity sufficient to reduce the proliferation of N. caninum in the brains of immunised mice.


A real-time quantitative PCR using the TaqMan fluorogenic detection system (TaqMan PCR) was established for identification of Ehrlichia risticii, the agent of Potomac horse fever (PHF). The TaqMan PCR identified an 85 base pair section of the 16S rRNA gene by use of a specific fluorogenic probe and two primers. This technique was specific for eight tested E. risticii strains. The TaqMan system identified 10 copies of a cloned section of the 16S rRNA gene of E. risticii. The sensitivity and specificity of the TaqMan PCR were similar to those of conventional nested PCR. The TaqMan PCR was evaluated on horses with infectious colitis and on freshwater stream snails collected from regions with a history of PHF. E. risticii could be detected in 22 of 153 (14.4%) horses with infectious colitis and in 25 of 234 (10.7%) snails in the TaqMan PCR. The same results were obtained in the conventional nested PCR. The Ehrlichia-load was in the range of 10,000-9,000,000 and 35,000-680,000,000 Ehrlichia equivalents per [mu]g leukocyte DNA and
snail DNA, respectively.


http://www.sciencedirect.com/science/article/B6TD7-3W379CH-4/2/221d06b725db203b5af5e9dc98f9272b

Fasciolosis is an economically significant disease of ruminants, caused by infection with the digenetic trematodes, Fasciola hepatica and F. gigantica. Some vaccination trials using irradiated metacercariae or isolated proteins have been shown to afford significant protection. However, the mechanisms of specific immunity against this pathogen have not been elucidated. We have identified thioredoxin, a tegument antigen of F. hepatica, among several proteins that are common to both the juvenile and adult fluke within the mammalian host and have undertaken studies to characterize bovine T cell responses to recombinant thioredoxin protein (FH 2020). Peripheral blood mononuclear cells from immune cattle proliferated specifically to crude F. hepatica antigenic extract but not to FH 2020. However, after repeated stimulation of lymphocytes by alternating crude extract and FH 2020, FH 2020-specific proliferation by T cell lines was observed. T cell clones were subsequently generated and found to respond specifically but weakly to both crude antigen and FH 2020. Thioredoxin appears to be only weakly antigenic for bovine T cells and is, therefore, an unpromising candidate for inducing resistance to F. hepatica.


http://www.sciencedirect.com/science/article/B6TD7-435CRSD-M/2/92a50bc7dcb423e443a5531c25592732

Nematode nicotinic acetylcholine receptors (nAChRs) are the sites of action for the anthelmintic drug levamisole. Recent findings indicate that the molecular mechanism of levamisole resistance may involve changes in the number and/or functions of target nAChRs. Accordingly, we have used an RT-PCR approach to isolate and characterise partial cDNA clones (tca-1 and tca-2) encoding putative nAChR subunits from the economically important trichostrongyloid, Teladorsagia circumcincta. The predicted tca-1 gene product is a 248 aa fragment (TCA-1) which contains structural motifs typical of ligand-binding ([alpha]-) subunits, and which shows very high sequence similarities (98.8 and 97.2% amino acid identities) to the [alpha]-subunits encoded by tar-1 and hca-1 from Trichostrongylus colubriformis and Haemonchus contortus, respectively. Sequence analyses of partial tca-1 cDNAs from one levamisole-resistant and two susceptible populations of T. circumcincta revealed polymorphism at the predicted amino acid level, but there was no apparent association of any particular tca-1 allele with resistance. tca-2 encodes a 67 aa fragment (TCA-2) containing the TM4 transmembrane domain and carboxyl terminus of a putative nAChR structural (non-[alpha]) subunit. The deduced amino acid sequence of TCA-2 shows highest similarity (75% amino acid identity) to ACR-2, a structural subunit involved in forming levamisole-gated ion channels in Caenorhabditis elegans, but low similarity (43% identity) to the corresponding regions of TAR-1 and HCA-1. tca-2 is the first nAChR subunit gene of this type to be isolated from parasitic nematodes, and it provides a basis for further characterisation of structural subunits in trichostrongyloids.
Three anthelmintic classes with distinct mechanisms of action are commercially available. Selection of nematode populations resistant to all these drugs has occurred, particularly in trichostrongyloid parasites of sheep. Anthelmintic resistance in cattle parasites has only recently been recognized and appears to be less pronounced, even though very similar species infect both hosts. To understand the bases for differences in the rate of resistance development in sheep versus cattle parasites, it is important to first demonstrate that the same kinds of resistance alleles exist in both. The benzimidazoles (BZ), which have been used for more than 40 years, were chosen as an example. BZ-sensitive (BZS) and BZ-resistant (BZR) nematodes that parasitize sheep have been distinguished at the molecular level by a single nucleotide change in the codon for amino acid 200 of a [beta]-tubulin gene, a switch from TC (phenylalanine) to TC (tyrosine). PCR primers were designed to completely conserved regions of trichostrongyloid [beta]-tubulin genes and were used to amplify DNA fragments from Haemonchus contortus (cDNA from a BZS and a BZR library) as positive controls. The technique was then extended to the cattle parasites, Cooperia oncophora and Ostertagia ostertagi (from genomic DNA). Sequence analysis proved the presence of amplified BZS alleles in all three species and BZR alleles in the BZR population of H. contortus. Based on these data, nested PCR primers using the diagnostic or as the most 3' nucleotide were designed for each species. Conditions for selective PCR were determined. To demonstrate feasibility, genomic DNA was recovered from individual H. contortus L3 larvae from both BZS and BZR populations. Genomic DNA was also isolated from >70 individual adult male C. oncophora collected from a cattle farm in New Zealand with reported BZ resistance. Allele-specific PCR discriminated among heterozygotes and homozygotes in both species. This method could find utility in studying the molecular epidemiology of BZ resistance in cattle parasites and for defining the variables that limit the development and spread of anthelmintic resistance in this host.

Vision Research 12


We screened 150 male eye donors and identified four who did not have or express L pigment genes, consistent with each of them having a congenital protan color vision defect. One donor was identified as a protanope because he had and expressed a single X-chromosome photopigment gene that encoded an M pigment. Three were categorized as protanomalous because each expressed significant levels of genes specifying two spectrally different M pigments. The first gene in each of the protanomalous arrays was expressed the most and encoded an M pigment that differed in amino acid sequence from M pigments in color normal men.

http://www.sciencedirect.com/science/article/B6T0W-454J5RW-8/2/546095c9d28979c46b82c4a0cd99f3af


http://www.sciencedirect.com/science/article/B6T0W-3YVBBWX-2/2/a3a1386f42d51b340927bb5db8740607

A novel cDNA encoding [alpha] subunit of the GTP-binding protein, transducin, has been cloned from a marine fish, Sparus aurata. The cDNA contains an open reading frame of 1050 nt (encoding 350 amino acid residues). A high degree of identity was found with known mammalian transducin proteins of cones (Gt2[alpha]) or rods (Gt1[alpha]): human Gt2[alpha] (80.2%), bovine Gt2[alpha] (79.3%), mouse Gt1[alpha] (78.2%), mouse Gt2[alpha] (78%) and bovine Gt1[alpha] (77.9%). Northern blot analysis of different tissues revealed a transcript of about 2.5 kb, which is expressed only in the fish eye and not in other tissues from adult fish, supporting its identification as transducin. Ontogeny of transducin mRNA expression during early development of Sparus aurata, determined by Northern blot analysis, showed very low levels in larvae 3 days after hatching but not earlier. Levels increased 3- and 6-fold on days 4 and 6 (respectively) compared with those on day 3 and remained essentially unchanged thereafter, until day 21 after hatching (the last day studied). Our results suggest that in fish only one [alpha] subunit of transducin is found, which shows similar identity with cone and rod [alpha] subunits of mammals.


http://www.sciencedirect.com/science/article/B6T0W-43N89PX-W/2/5cf6d66a7d142b3c688cd2bfcf986e60e

Females heterozygous for congenital colour vision defects are of interest because they are believed to have cone photoreceptor ratios and cone photopigments that differ from normal. We describe a molecular genetic method to identify protan carriers that involves characterizing the genes that occur in the most upstream position in each of the X-chromosome photopigment gene arrays.


http://www.sciencedirect.com/science/article/B6T0W-3TGVGKR-1/2/320bc635a4622e7a2f97906a495e5679

Variations in the amino acid sequences of the human cone opsins give rise to spectrally variant subtypes of L and M cone pigments even in the population with normal color vision. In vitro mutagenesis studies have shown that a limited number of amino acid substitutions produce shifts
in the wavelength sensitivity. Presented here are results comparing electrophysiological measurements of single human cones with the expressed cone pigment gene sequences from the same retina. In a sample of eight long-wavelength sensitive cone (L cone) spectra obtained from five donors the precise spectral sensitivities, measured in situ, of the two most commonly occurring spectral variants were determined. The peak sensitivity of the Lser180 cone was 563 nm while that of the Lala180 cone was 559 nm.


Photoreceptor cGMP phosphodiesterases (PDE6 family) are modular enzymes with each catalytic subunit containing two N-terminal regulatory GAF domains, GAF A and GAF B. The GAF A domains contribute to dimerization of the PDE6 catalytic subunits and to binding of the inhibitory P[gamma] subunits, and represent candidate sites for noncatalytic binding of cGMP. We performed a mutational analysis of selected residues from the GAF A domain of cone PDE[alpha] to identify the cGMP-binding pocket and delineate the P[gamma]-binding surface. Results of this analysis establish the noncatalytic cGMP-binding site within the PDE6 GAF A domain and suggest that occupation of the pocket by cGMP is required for high-affinity binding of P[gamma] to the proximate contact surface.


http://www.sciencedirect.com/science/article/B6T0W-4CX0S4Y-1/2/93ac035ef300ef22df1024ae946c14dd

Photoreceptor cGMP phosphodiesterases (PDE6 family) are modular enzymes with each catalytic subunit containing two N-terminal regulatory GAF domains, GAF A and GAF B. The GAF A domains contribute to dimerization of the PDE6 catalytic subunits and to binding of the inhibitory P[gamma] subunits, and represent candidate sites for noncatalytic binding of cGMP. We performed a mutational analysis of selected residues from the GAF A domain of cone PDE[alpha] to identify the cGMP-binding pocket and delineate the P[gamma]-binding surface. Results of this analysis establish the noncatalytic cGMP-binding site within the PDE6 GAF A domain and suggest that occupation of the pocket by cGMP is required for high-affinity binding of P[gamma] to the proximate contact surface.


http://www.sciencedirect.com/science/article/B6T0W-43N89PX-4/2/a71c6582c4c5e8ac8eef6d4003a35a4e

Spectral subtypes of L pigment are produced by a serine/alanine dimorphism at amino acid position 180. X-chromosomes that carry genes for the different subtypes occur with about equal frequency in normal men. Females have two X-chromosomes; thus, about 50% of women will inherit genes for both L pigment subtypes, although on different X-chromosomes. In these women, X-inactivation is expected to produce about equal numbers of LS180 and LA180 cones in
addition to middle (M) and short (S) wavelength-sensitive cones to total four spectrally distinct cone types. Consistent with this expectation we found nearly equal expression of genes for two spectrally distinct subtypes of L pigment in five of nine female retinas examined.


http://www.sciencedirect.com/science/article/B6T0W-3XD3H6N-3/2/c39b907ec0d63957bafa23a1c5a91a85

Carotid artery occlusion (two vessel occlusion; 2-VO) for 3 or 9 months causes a suppression of the electroretinogram. However, after 3 months the retinal morphology appears unaffected judging from the localisation of GABA, ChAT, [alpha]PKC, Thy-1 and GFAP immunoreactivities. Moreover, no difference in NMDA-R1, opsin or Thy-1 mRNA levels were detected. In contrast, after 9 months 2-VO photoreceptor degeneration occurred as indicated by thinning of the outer nuclear layer and reduced Ret-P1 immunoreactivity. All other immunoreactivities appeared normal. These findings were supported by analysis of retinal mRNA levels. We conclude that the major effect of prolonged 2-VO is photoreceptor degeneration.


http://www.sciencedirect.com/science/article/B6T0W-43N89PX-3/2/c28ebef78b26eab0aef85f1fa8620f4

To directly test the hypothesis that only two pigment genes are expressed from the X-chromosome array, we examined expressed M and L pigment gene sequences from >100 male eye donors. In this sample, there were eight men who expressed high levels of more than one L pigment gene in addition to M pigment genes. The fact that these eyes expressed both L and M pigment genes at significant levels suggests they were from men with normal colour vision. We reject the hypothesis that only two pigment genes from one X-chromosome array can be expressed.


http://www.sciencedirect.com/science/article/B6T0W-4F1SVBD-9/2/0330fcd6e256125cb6d904166c842d52e

We determined the structures of long (L)-wavelength-sensitive and middle (M)-wavelength-sensitive opsin gene array of 58 male chimpanzees and we investigated relative sensitivity to red and green lights by electroretinogram flicker photometry. One subject had protanomalous color vision, while others had normal color vision. Unlike in humans, a polymorphic difference in the copy number of the genes and a polymorphic base substitution at amino acid position 180 were not frequently observed in chimpanzees.

The cone 'synaptic complex' is a unique structure in which a single presynaptic axon secretes glutamate onto processes of bipolar cells (both ON and OFF) and horizontal cells. In turn, the horizontal cell processes antagonize cone and bipolar responses to glutamate (probably by GABA). What still remains largely unknown is the molecular identity of the postsynaptic receptors and their exact locations. We identified several subunits of the glutamate receptor and the GABAA receptor expressed at the cone synaptic complex and localized them ultrastructurally.

Glutamate receptors: (i) Invaginating (probably ON) bipolar dendrites in the monkey and rat express the metabotropic glutamate receptor, mGluR6. The stain is intense on the dendritic membrane where it first enters the invagination, and weak at the tip nearest to the ribbon. The cone membrane is electron-dense where it apposes the intense stain for mGluR6. Surprisingly, invaginating bipolar dendrites in the cat also express the AMPA receptor subunits, GluR2/3 and GluR4. (ii) Dendrites forming basal contacts in the cat (probably OFF) express the AMPA subunits GluR2/3, GluR4, and also the kainate subunit, GluR6/7. The stain is especially intense at the dendritic tips in apposition to electron-dense regions of cone membrane. (iii) Horizontal cells in the cat express the AMPA subunits GluR2/3, GluR4 and the kainate subunit, GluR6/7. The stain is strongest in the cytosol of somas and primary dendrites, but is also present in the invaginating terminals where it localizes to the membrane subjacent to the ribbon. GABAA receptors: (i) ON and OFF bipolar dendrites in the monkey express the [alpha]1 and [beta]2/3 subunits. The stain is localized to the bipolar cell membrane in apposition to horizontal cell processes. (ii) Cones did not express the GABAA subunits tested by immunocytochemistry, but [beta]3 mRNA was amplified by RT-PCR from rat photoreceptors. Conclusions: (i) mGluR6 receptors concentrate on dendrites at the base of the invagination rather than at the apex. This implies that receptors at both 'invaginating' and 'basal' contacts lie roughly equidistant from the release sites and should therefore receive similar spatiotemporal concentrations of glutamate. (ii) The 'cone' membrane is electron-dense opposite to the receptor sites on both ON and OFF bipolar cells. This suggests a special role for this region in synaptic transmission. Possibly, these densities signify a transporter that would regulate glutamate concentration at sites remote (>200 nm) from the locus of vesicle release.

A small study was undertaken to examine the microbiological characteristics of spent mushroom compost (SMC) waste by DNA sequence typing: ecological considerations of disposal on agricultural land. A small study was undertaken to examine the microbiological characteristics of spent mushroom compost (SMC), which is the major waste by-product of the mushroom industry and which is regularly disposed off by application to agricultural land. The primary aim of this study was to examine SMC for the presence of faecal bacterial pathogens, including Campylobacter spp., Salmonella spp. and Listeria monocytogenes. Secondly it was desirable to quantify bacterial and fungal populations within SMC, and also qualitatively identify the diversity of bacterial populations...
within SMC, through employment of rDNA PCR and direct sequencing techniques on the culturable microflora. Conventional microbiological analyses of SMC material (n=30) from six commercial operations in both Northern Ireland and the Republic of Ireland, failed to detect Salmonella spp, Listeria spp. or Campylobacter spp. in any of the SMC material examined. Total aerobic plate counts gave a mean count of log10 7.01 colony forming units (cfu) per gram SMC material (range: log10 6.53-7.52 cfu/g). Fungal counts gave a mean count of log10 4.57 cfu per gram SMC material (range: log10 3.93-4.98 cfu/g). From a total of greater than 50 colony picks, a total of 12 bacterial morphotypes were identified and were further examined by employment of partial 16S rRNA gene amplification and sequencing techniques, yielding several genera and species, including Bacillus licheniformis, Bacillus subtilis, Klebsiella/Enterobacter sp. Microbacterium sp. Paenibacillus lentimorbus, Pseudomonas mevalonii, Sphingobacterium multivorum and Stenotrophomonas sp. This is the first preliminary report on the microbial diversity of SMC waste and demonstrates the presence of several species that have not been previously described in SMC, in addition to two potentially novel species within the genera Microbacterium and Stenotrophomonas. It is thereby important to examine the ecological microbe-microbe and plant-microbe interactions that are occurring between the native bacterial soil flora and those added annually (theoretically estimated at approximately 1018 cells) through the application of SMC. Such studies would be beneficial in helping to ascertain the ecological consequences involved in the disposal of SMC waste on agricultural land.

Water Research (9)


http://www.sciencedirect.com/science/article/B6V73-48PV2FF-R/2/e3e5f154b3bd9fe09e47f5998bc2dac2b

Although activated sludge systems with enhanced biological phosphorus removal (EBPR) represent state-of-the-art technology for phosphate removal from wastewater it is still unknown which species of bacteria are responsible for the EBPR process. The aim of this study was to compare the bacterial composition of activated sludge from two laboratory plants with different modes of operation, anoxic/oxic- (EBPR, no nitrification) and Phoredox-system (EBPR, nitrification and denitrification) with particular emphasis on microorganisms responsible for EBPR process. In addition to fluorescence in situ hybridization (FISH), we applied further rRNA-based molecular techniques like terminal restriction-fragment length polymorphism analysis and comparative 16S rDNA analysis to yield additional information and to verify the results from FISH analysis, like e.g. for the identification of polyphosphate accumulating organisms (PAO). Despite the different modes of operation only minor differences in the bacterial composition were detected by FISH analysis based on the probes used in this study. In contrast T-RFLP analysis yielded characteristic community fingerprints for each of the investigated plants and comparative 16S rDNA analysis indicated highly diverse microbial communities in both plants suggesting substantial differences in the microbial structure. The results obtained by FISH analysis with specific probes for PAOs support the presumption that not only one specific organism is responsible for the EBPR. In our case Tetrasphaera spp. dominated the PAO community, but other possible PAOs, like Microlunatus spp. and members of the Rhodocyclus group, were also detected.

http://www.sciencedirect.com/science/article/B6V73-4F3FSB2-4/2/08d994854cce2a834dbb6da18d69379d

Cell densities of the fecal pollution indicator genus, Enterococcus, were determined by a rapid (3 h or less) quantitative polymerase chain reaction (QPCR) analysis method in 100 ml water samples collected from recreational beaches on Lake Michigan and Lake Erie during the summer of 2003. Measurements by this method were compared with counts of Enterococcus colony-forming units (CFU) determined by Method 1600 membrane filter (MF) analysis using mEI agar. The QPCR method had an estimated 95% confidence, minimum detection limit of 27 Enterococcus cells per sample in analyses of undiluted DNA extracts and quantitative analyses of multiple lake water samples, spiked with known numbers of these organisms, gave geometric mean results that were highly consistent with the spike levels. At both beaches, the geometric means of ambient Enterococcus concentrations in water samples, determined from multiple collection points during each sampling visit, showed approximately lognormal distributions over the study period using both QPCR and MF analyses. These geometric means ranged from 10 to 8548 cells by QPCR analysis and 1-2499 CFU by MF culture analysis in Lake Michigan (N=56) and from 8 to 8695 cells by QPCR and 3-1941 CFU by MF culture in Lake Erie (N=47). Regression analysis of these results showed a significant positive correlation between the two methods with an overall correlation coefficient (r) of 0.68.


http://www.sciencedirect.com/science/article/B6V73-44HTG7C-7/2/917252f0590000e2f10cdf5df86ec190

Microbial community structure of a lab scale thermophilic aerobic wastewater treatment reactor was analyzed by a combination of culture-independent methods. Quinone profile method provides for chemical analysis of respiratory quinone molecular species, which corresponds to bacterial groups. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA partial sequences (PCR-DGGE) clarifies community changes at species level, as DGGE can separate DNA fragments of different sequences. Certain phylogenetic groups of bacterial cells can be labeled by fluorescence in situ hybridization (FISH). Quinone profile showed a predominant presence of MK-7. PCR-DGGE revealed that constituents of the community were unchanged during the stable phase. FISH demonstrated the existence of the relatives of Bacillus lentus and B. thermocloacae in considerable proportions. The community was mainly composed of Bacillaceae, and obligate thermophilic and mesophilic Bacillus appeared in spite of the temperature fluctuation from 35[deg]C to 60[deg]C. The combination of these culture-independent methods revealed the community precisely enough to evaluate the reactor performance.


http://www.sciencedirect.com/science/article/B6V73-4DFK5TH-2/2/8b173bfd80775c3c461e39f608a55749
2-Methylisoborneol (MIB), a metabolite of blue-green algae, has been implicated in causing unpalatable drinking water throughout the world. Current non-biological water treatment technologies are ineffective in removing MIB from potable water or are cost-prohibitive, and biological applications may address these problems. We have isolated and characterized a bacterium derived from lake water and capable of aerobically degrading MIB. Light microscopy and transmission electron microscopy revealed that this strain is a spore-forming, flagellated bacterium that is bacilloid in shape, and 16S rRNA phylogenetic analysis determined that it is most closely related to Bacillus fusiformis and Bacillus sphaericus, both members of the Bacillus sphaericus sensu lato taxon. While the growth and oxidation potential of this strain was shown to be affected beyond certain MIB concentrations in the mg/l range, it was capable of depleting MIB at mg/l and ng/l concentrations and of removing MIB to concentrations yielding no observed odor.


http://www.sciencedirect.com/science/article/B6V73-42K5F1M-3/2/4151e10063c06eae601851601a1eb124

A nested-PCR assay, incorporating an internal positive control, was developed for Cryptosporidium monitoring in finished water. This assay was capable of reproducibly detecting 8 oocysts in spiked-filtered water samples collected from 5 South Australian water treatment plants. The RT-PCR assay of Kaucner and Stinear (Appl. Environ. Microbiol. 64(5) (1998) 1743) was also evaluated for the detection of Cryptosporidium parvum. Initially, under our experimental conditions, a detection level of 27 oocysts was achieved for spiked reagent water samples. This level was improved to 5 oocysts by modification of the method. Untreated South Australian source waters concentrated by calcium carbonate flocculation were found to be highly inhibitory to the RT-PCR assay. Concentration of similar samples using Envirocheck filters appeared to eliminate PCR inhibition. While both methods possessed similar sensitivities the nested-PCR assay was more reproducible, more cost effective, simpler to perform and could detect both viable and non-viable intact Cryptosporidium parvum oocysts, which is an important consideration for plant operators. These factors make the nested-PCR assay the method of choice for screening large numbers of potable water samples, where a reliable low level of detection is essential.


http://www.sciencedirect.com/science/article/B6V73-3YKKH8C-8B/2/28d09f93272fc5d2461720807bcc92fc

Fifteen enteroviruses (EVs) previously isolated from Tyrrhenian sea water samples were used. They were first identified by traditional dot-blot and Northern-blot hybridizations with a group of cDNA probes from cloned Poliovirus 1 and Coxsackievirus B4 and oligodeoxynucleotides complementary to echovirus 6 and 9 sequences. Using both wild viruses and known enteroviruses a reverse-PCR protocol was then set up followed by cDNA sequencing of the fragments generated. The sequences of primers were selected from a consensus of several 5' non-coding ends of enterovirus genomes, representing highly conserved regions. The downstream (region 577-603) and the upstream (region 436-465) oligonucleotide primers carried an extra sequence in order to generate BamHI and a HindIII restriction sites at the 5' and 3' end respectively of the amplified cDNA fragments for directional cloning in a plasmid. The downstream 5'NC primer was 5'-biotinylated in order to allow direct sequencing of the amplicon,
when possible, after strand separations on streptavidin coated magnetic beads. The PCR of reverse transcribed viral RNAs resulted in a 167-170 b.p. cDNA product on ethidium bromide-stained 2% agarose gels in all the samples and reference viruses. The test is negative on reoviruses, hepatitis A and uninfected BGM cells and detects 50 viral particles. Sequences of cloned fragments were compared with sequences of cloned enteroviruses stored in commercial data banks. The 5'NC region of a reference echovirus 5 was also cloned and sequenced to improve the comparison. On the basis of deduced genetic distances, three poliovirus 1, eight coxsakievirus B5, four coxsakievirus B1 were diagnosed. One poliovirus Sabin 2 was isolated together with a coxsackievirus-related strain in the same lysate sample. The reliability and sensitivity of this RT-PCR method makes it an attractive approach to virus detection in environmental samples.


http://www.sciencedirect.com/science/article/B6V73-3X70S5S-G/2/d5aa42adb731dfdc0062135f1284c323

In a previous study [Muscillo, M., Carducci, A., La Rosa, G., Cantiani, L., Marianelli, C. (1997a) Enteric virus detection in adriatic seawater by cell culture, polymerase chain reaction and polyacrylamide gel electrophoresis. *Water Res.* **31**, 1980-1984] enterovirus strains were isolated from Adriatic seawater and estuarine water from the Foglia River, by infecting susceptible cells with ultrafiltrated water samples. In the present work we have studied three of those samples, in which routine reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing analysis had identified the presence of poliovirus type 3 (P3). In order to better estimate the risk to human health of such occurrence in bathing water (having bacteriological standards in line with the EEC directive 76/160), we set up a protocol to distinguish wild from Sabin P3 strains. Three sets of RT-PCR primers were engineered and their predicted products were: 593 nucleotides (nt) in the 5' noncoding (5'NC) region (11-603), 350 nt at the Vp3-Vp1 junction (2438-2787) of the capsid protein genes, and 420 nt in the 2C (4209-4628) region, which is regarded as the hotspot of recombinant polioviruses. Eight reference ATCC strains, whose sequences were known, were also tested under the same experimental conditions in order to verify the accuracy of the RT-PCR reactions. The amplicons were directly sequenced by Big-dye(TM) terminator sequencing using a capillary automatic sequencer. The latter two regions found the same viral species Polio 3 in all the sample strains, with no meaningful distinction between P3/Leon/37 and P3/Leon/12a1b, the vaccine strain. The analyses in the 5'NC region were more useful, where genetic relationships and the predicted secondary structure suggested that the viruses were of vaccinal sources. Molecular data were confirmed by in vitro phenotypic marker tests rct/40, where all the examined samples displayed a temperature sensitive phenotype rct/40(-). Our results suggest that the 472U->C transition alone, is not a predictive marker of reversion to neurovirulence. Finally, we conclude that the 220U constantly found in the consensus sequences of the samples can serve as a good predictor of rct/40(-) phenotype.


http://www.sciencedirect.com/science/article/B6V73-41S4TCG-X/2/0d87a7d0ddaaf1c88bf0a2a298246f5ec

The frequent occurrence of reoviruses in environmental samples could be a potential source of interference with enterovirus detection, especially when enterovirus isolation on cell culture is required. In order to evaluate new virus-based criteria for enforcing recreational water quality
standards, a new method based on a broad reverse transcribed polymerase chain reaction (RT-PCR) was set up to detect reoviruses. Two primers were engineered to amplify a 538 base pair fragment of the Sigma 2 gene. Reovirus strains obtained from ATCC (Jones, Lang, Dearing, Abney, NC-TEV, SV59 and SV12) were used as references. Twenty-four samples of 10 l were collected from two beaches of the Adriatic sea and 12 from the neighbourhood of Fano Harbour Channel. The presence of environmental reoviruses was tested on both concentrated seawater samples and lysates of BGM cells infected with the concentrated seawater samples. The new method was used in parallel with the detection of a 3: 3: 4 electrophoretic pattern of reovirus RNA in polyacrylamide gel electrophoresis (PAGE). Enterovirus and bacteria were also screened in compliance with EEC directives. No enteroviruses were isolated, and it was not attributable to reovirus interference. All the reovirus found by PAGE (8/72) were confirmed by RT-PCR, while several genomes (14/72) were detected only by RT-PCR. Presumptive methods of virus identification, that is CPE on BGM cells and haemagglutination test, were not able to detect them. The specificity of RT-PCR products was checked by direct nucleotide sequence analyses of the amplicons. The phylogenetic analyses showed heterogeneous taxa including human and animal reoviruses, with strong evidence that they were spreading consistently from the Harbour-Channel. This novel approach for reovirus detection will be very useful as a trace route of faecal pollution; more importantly, it could be very useful in contributing to the creation of a databank of circulating enteric viruses.


We demonstrated previously that micro-aeration allows construction of an effective thermophilic methane-fermentation system for treatment of municipal solid waste (MSW) without production of H2S. In the present study, we compared the microbial communities in a thermophilic MSW digester without aeration and with micro-aeration by fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), phylogenetic analysis of libraries of 16S rRNA gene clones and quantitative real-time PCR. Moreover, we studied the activity of sulfate-reducing bacteria (SRB) by analysis of the transcription of the gene for dissimilatory sulfite reductase (dsr). Experiments using FISH revealed that microorganisms belonging to the domain Bacteria dominated in the digester both without aeration and with micro-aeration. Phylogenetic analysis based on 16S rRNA gene and analysis of bacteria by DGGE did not reveal any obvious difference within the microbial communities under the two aeration conditions, and bacteria affiliated with the phylum Firmicutes were dominant. In Archaea, the population of Methanosarcina decreased while the population of Methanoculleus increased as a result of micro-aerations as revealed by the analysis of 16S rRNA gene clones and quantitative real-time PCR. Reverse transcription and PCR (RT-PCR) demonstrated the transcription of dsrA not only in the absence of aeration but also in the presence of micro-aeration, even under conditions where no H2S was detected in the biogas. In conclusion, micro-aeration has no obvious effects on the phylogenetic diversity of microorganisms. Furthermore, the activity of SRBs in the digester was not repressed even though the concentration of H2S in the biogas was very low under the micro-aeration conditions.